J. Pineal Res. 2014; 57:131–146
Doi:10.1111/jpi.12162

Journal of Pineal Research

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

REVIEW ARTICLE

Melatonin: a well-documented antioxidant with conditional pro-oxidant actions

Abstract: Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine produced in many organs including the pineal gland, was initially characterized as a hormone primarily involved in circadian regulation of physiological and neuroendocrine function. Subsequent studies found that melatonin and its metabolic derivatives possess strong free radical scavenging properties. These metabolites are potent antioxidants against both ROS (reactive oxygen species) and RNS (reactive nitrogen species). The mechanisms by which melatonin and its metabolites protect against free radicals and oxidative stress include direct scavenging of radicals and radical products, induction of the expression of antioxidant enzymes, reduction of the activation of pro-oxidant enzymes, and maintenance of mitochondrial homeostasis. In both in vitro and in vivo studies, melatonin has been shown to reduce oxidative damage to lipids, proteins and DNA under a very wide set of conditions where toxic derivatives of oxygen are known to be produced. Although the vast majority of studies proved the antioxidant capacity of melatonin and its derivatives, a few studies using cultured cells found that melatonin promoted the generation of ROS at pharmacological concentrations (μM to mM range) in several tumor and nontumor cells; thus, melatonin functioned as a conditional pro-oxidant. Mechanistically, melatonin may stimulate ROS production through its interaction with calmodulin. Also, melatonin may interact with mitochondrial complex III or mitochondrial transition pore to promote ROS production. Whether melatonin functions as a pro-oxidant under in vivo conditions is not well documented; thus, whether the reported in vitro pro-oxidant actions come into play in live organisms remains to be established.

Hong-Mei Zhang[1] and Yiqiang Zhang[2,3]

[1]Department of Clinical Oncology, Xijing Hospital, The Fourth Military Medical University, Xi'an, China; [2]Barshop Institute, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [3]Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Key words: antioxidant, free radicals, melatonin, mitochondria, pro-oxidant, reactive nitrogen species, reactive oxygen species

Address reprint requests to Hong-Mei Zhang, Department of Oncology, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032, China.
E-mail: zhm@fmmu.edu.cn and
Yiqiang Zhang, Barshop Institute for Longevity and Aging Studies & Department of Physiology, The University of Texas Health Science Center at San Antonio, 15355 Lambda Drive, San Antonio, TX 78245, USA.
E-mail: zhangy@uthscsa.edu

Received July 8, 2014;
Accepted July 18, 2014.

Introduction

Melatonin is an evolutionally phylogenic old molecule, which can be traced back to the ancient photosynthetic prokaryotes. It is a tryptophan derivative that was first isolated from bovine pineal glands [1]. Melatonin was later found to be also present or synthesized in extrapineal tissues such as retina, Harderian gland, gastrointestinal tract, testes and lymphocytes [2]. Melatonin is a functionally diverse molecule [3]; its originally described mission was the regulation of circadian and circannual cycles [4–7]. These actions directly involve membrane receptors specific for the indoleamine situated in the germane tissues. More recently, melatonin and its derivatives were found to be potent free radical scavengers and broad-spectrum antioxidants, properties that have been conserved through evolution [8, 9]. Mechanistically, melatonin executes its free radical scavenging actions via nonreceptor-mediated processes, while its stimulation of antioxidant enzymes involves receptors [10, 11]. In mammals, melatonin activates at least two high-affinity G protein-coupled receptors, the MT1 and MT2, which regulate a variety of cellular and physiological processes including neuronal firing, arterial vasoconstriction, cell proliferation, immune responses, and reproductive and metabolic functions [12, 13]. Aside from MT1 and MT2 receptors, melatonin has been shown to interact with other intracellular proteins, including nuclear receptor ROR/RZR, quinone reductase 2 (or MT3), and calmodulin [3]. ROR/RZR has been proposed to work in coordination with the plasma membrane receptors MT1/MT2 to regulate gene expression [14]. The interaction between melatonin and quinone reductase is possibly involved in the regulation of cellular redox status, although the exact role of this interaction remains poorly understood [15]. The low-affinity interaction between melatonin and calmodulin may be involved in its antioxidant action as well as other signaling processes [16].

Although the vast majority of experiments have documented that melatonin is a powerful free radical scavenger and antioxidant, some recent in vitro evidence suggests that melatonin may have pro-oxidant actions in some cells [17–28]. Most notably, melatonin functions as a pro-oxidant in cancer cells where it aids in the killing of tumor cells [18, 19, 21, 25, 27, 28]. In the following discussion, we

will review the published studies and present arguments to reconcile the two seemingly opposing actions of melatonin with regard to its relationship with free radicals.

Free radicals

Free radicals are continuously produced in aerobic organisms as byproducts from oxidative reactions [29]. Two groups of toxic species, some of which are free radicals, have been extensively studied for their roles in diseases and aging: reactive oxygen species (ROS) and reactive nitrogen species (RNS). Common ROS includes singlet oxygen ($^1O_2$), superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$), with the latter being the most reactive and doing the largest amount of damage in living cells. Oxidants are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as wel as from a variety of cytosolic enzyme systems (e.g. lipoxygenases, NADPH oxidase, and cytochrome P450) [30]. In addition, a number of external agents can trigger ROS production (e.g. ionizing radiation, ultraviolet light, environmental toxins, inflammatory and cytokines). The mitochondrion is a major site of $O_2^{\cdot -}$ generation during oxidative phosphorylation. $O_2^{\cdot -}$ is rapidly converted to H_2O_2 through enzymatic reactions catalyzed by the superoxide dismutases (SODs) localized in different cellular compartments (mitochondria, cytosol, and extracellular). H_2O_2 can subsequently be metabolized to water or to the devastatingly toxic $^{\cdot}OH$. The $^{\cdot}OH$ is formed during the Fenton reaction when H_2O_2 interacts with transition metals (Fe^{2+}, Cu^{1+}, etc.). RNS includes nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$), and peroxynitrate (ONOOH), with peroxynitrite being the most toxic RNS. $NO^{\cdot}$ is generated from L-arginine catalytically and involves nitric oxide synthase [31, 32]. $NO^{\cdot}$ can subsequently couple with $O_2^{\cdot -}$ to generate $ONOO^-$. This product eventually degrades into ONOOH and $^{\cdot}OH$ or $^{\cdot}OH$-like products.

Free radicals function as double-edged swords in biological systems as they can be either harmful or beneficial to living systems, depending on the site of generation and their levels [33]. Generally, in high concentrations, ROS/RNS are important mediators of damage to cellular components (e.g. lipids, proteins, and DNA). The deleterious consequences of free radical damage, referred to as oxidative stress, at cellular level can be cell death, senescence or tumorigenesis. Free radicals promote cytochrome c release from mitochondria, activation of caspases, cell cycle control protein p53, and other apoptotic signaling proteins in a mitochondria-dependent or mitochondria-independent manner [34, 35]. The destructive effects of free radicals are associated with the induction of cellular senescence [36], and they play important roles in cancer initiation and progression [37].

To defend against free radical damage, cells have developed multiple systems to keep levels of free radicals in check. These include oxidant scavengers to directly neutralize free radicals and antioxidant enzymatic systems to convert toxic free radicals to nontoxic molecules. In addition, the cells have developed systems to repair or clear molecules or structures damaged by free radicals. For instance, oxidatively damaged DNA can be repaired by base excision repair system, while oxidatively damaged proteins are removed by autophagy [38, 39]. Oxidative stress occurs when the levels of free radicals overwhelm the cellular defense system designed to metabolize them. The resulting damage leads to the interruption of normal cellular operations with potentially disastrous physiological consequences.

Numerous studies have also revealed the beneficial effects of ROS/RNS on the host [40]. ROS/RNS are powerful agents against infectious agents and function in the first line of defense against infection [41]. Oxidative/respiratory burst occurs during phagocytosis of infectious agents by phagocytic cells (e.g. macrophages and neutrophils), thereby protecting against infectious agents [42, 43]. ROS/RNS are also critical secondary messengers for multiple cellular signaling pathways involved in cell growth, differentiation, and inflammatory responses [44, 45]. As an example, oxidation reduction of protein cysteine residues regulates signaling pathways through the activation or inactivation of proteins/enzymes [46]. Also, $NO^{\cdot}$ has been recognized to play an important role in the nitric oxide-cyclic GMP signal transduction pathway [47].

The roles of ROS/RNS in a living system are highly complex and usually well regulated. The balance between the deleterious and beneficial effects of ROS/RNS depends on multiple factors, including but not limited to the concentration of ROS/RNS, the cell type, the subcellular compartments where they are generated, and the timing of the generation.

Melatonin as an antioxidant

Melatonin and its derivatives have been shown to be powerful direct free radical scavengers [8, 9, 31, 48–52]. Several mechanisms by which melatonin detoxifies oxidants are considered to be operative, including single electron transfer, hydrogen transfer, and radical adduct formation [50, 51]. Besides direct scavenging of ROS/RNS, melatonin also stimulates antioxidant enzymes [10, 11, 53], suppresses pro-oxidant enzymes [53–55], and improves mitochondrial function thereby reducing radical formation [56–59] (Fig. 1).

Within the cell, the free radical scavenging capacity of melatonin could be affected by its subcellular localization [2]. Melatonin differentially distributes in various subcellular compartments in extra-pineal tissues. Menendez-Pelaez et al. [60, 61] first reported that melatonin was distributed to the nucleus. Recently, Venegas et al. [62] reported that melatonin levels in the cell membrane, cytosol, nucleus, and mitochondrion varied over a 24-hr cycle, with some evidence of rhythmicity. The cell membrane had the highest concentration of melatonin followed by mitochondria, nucleus, and cytosol. When administered in vivo, melatonin in doses ranging from 40 to 200 mg/kg body weight increased the accumulation of melatonin in cell membrane and cytosol in a dose-dependent manner, with values 100 times greater in the former than in the latter. Melatonin levels in the nucleus and mitochondria reached saturation with a lower dose of 40 mg/kg body weight, with no further accumulation under higher doses of injected melatonin. Notable is that Venegas et al. [62] observed that

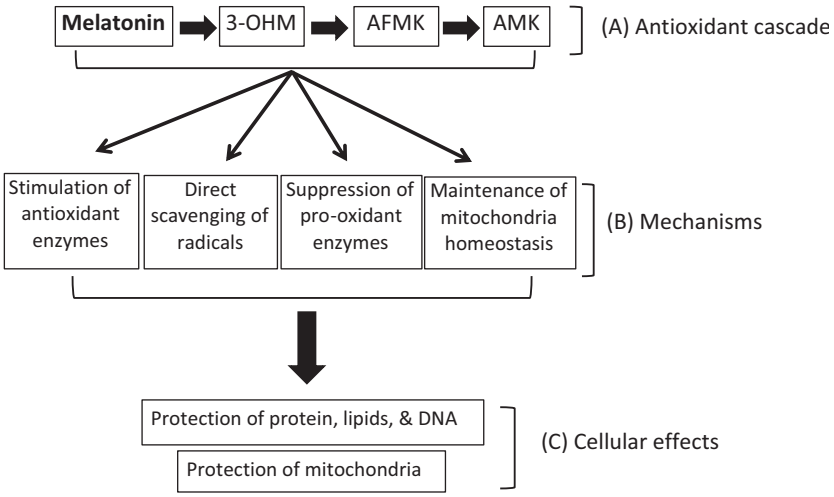

Fig. 1. Melatonin's antioxidant cascade and mechanisms of protection against oxidative damage. In this review, only the well-studied melatonin metabolites which have significant antioxidant actions are listed. The protection of mitochondria by melatonin and its metabolites is considered as an independent mechanism of cellular protection. However, as described in the text, the other three mechanisms may all be involved in maintaining mitochondrial homeostasis.

pinealectomy, which lowers blood melatonin values, rather than reducing mitochondrial concentrations of melatonin actually caused them to increase.

Direct scavenging of ROS

Melatonin was first demonstrated to be a direct scavenger of oxidants by Tan et al. [49]. Using an in vitro system, this group found that melatonin directly scavenged the highly toxic ·OH. Other studies subsequently confirmed this action of melatonin, using a variety of in vitro methodologies under well-controlled and sometimes cell-free conditions [63–69]. Li et al. [70] provided the first evidence that melatonin neutralized ·OH in vivo. In this study, administration of melatonin reduced ·OH generated during cerebral ischemia-reperfusion, a finding supported by the study of Bromme et al. [71].

When melatonin detoxifies the ·OH, it is converted to cyclic 3-hydroxymelatonin (3-OHM), which is also a potent radical scavenger [72–75]. 3-OHM is an intermediate metabolite of melatonin. It undergoes oxidation resulting in the formation of N^1-acetyl-N^2-formyl-methoxykynuramne (AFMK) [74]. Deformylation of AFMK leads to the generation of N2-acetyl-5-methoxykynuramine (AMK) [76, 77]. These compounds are also major melatonin metabolites with a similar potency to melatonin in detoxifying ROS and reducing oxidative stress [78–81]. The sequential scavenging of ROS by melatonin and its metabolites is known as melatonin's antioxidant cascade [82, 83] (Fig. 1).

The physicochemical nature of the reaction between melatonin and free radicals has been investigated. Three mechanisms have been proposed and tested, including single electron transfer, hydrogen atom transfer, and radical adduct formation [50, 84]. Using In vitro cell-free system in the presence of one-electron oxidants, $ONOO^-$, or alkoxyl radicals, several studies found that single electron transfer and hydrogen atom transfer were important characteristics of the antioxidant activity of melatonin [85–88]. In the presence of ·OH, however, radical adduct formation seems to be the major mechanism by which melatonin detoxifies ·OH [66].

Melatonin has also been shown to react in primarily in vitro cell-free systems with H_2O_2 [77, 89], $^1O_2$ [89–91], NO· [85, 92, 93], $ONOO^-$/ONOOH/ONOOH* [87, 88, 93, 94], and LOO· [95]. The capability of melatonin to directly detoxify $O_2^{\cdot-}$ and hypochlorous acid (HOCI) remains to be conclusively determined [31]. The in vitro findings that melatonin detoxifies $ONOO^-$ is consistent with the results of several in vivo studies, which demonstrated that melatonin administration in vivo reduced the accumulation of nitrotyrosine, a footprint of $ONOO^-$ nitration of tyrosine [96–99]. Also, Yin et al. [100] reported that melatonin arrested $ONOO^-$-induced tau hyperphosphorylation and the overactivation of protein kinases with the reduction of oxidative stress in rat brain.

Regulating the expression or activity of proteins

Melatonin and its metabolites have been demonstrated to modulate the expression of genes involved in detoxification or generation of ROS/RNS (for extensive discussion please refer to the reviews by Luchetti et al. [16] and Rodriguez and coworkers [53]). Antolin et al. [101] first demonstrated that melatonin enhanced the expression of genes involved in detoxifying ROS, including CuZnSOD (copper zinc superoxide dismutase, or SOD1) and MnSOD (manganese superoxide dismutase, or SOD2) in hamster Harderian gland. Mayo et al. [102] subsequently demonstrated that melatonin at a physiological serum concentration (1 nM) increased the expression of CuZnSOD and glutathione peroxidase (GPx) in neural cell lines. In contrast, Tang et al. [103] found that higher concentrations of melatonin (30–120 μM range) did not alter gene expression of CuZnSOD and GPx in neuronal cells. These results suggest that the melatonin affects the expression of antioxidative stress genes in a dose-dependent manner. The melatonin-mediated changes in antioxidant enzyme gene expression are consistent with melatonin's ability to upregulate the activities of these enzymes [10, 11, 53].

When it was administered in vivo, melatonin increased gene expression of multiple antioxidative stress genes in neuronal tissues, including CuZnSOD, MnSOD, GPx, catalase, and glutathione reductase (GR) [104–106].

Melatonin, when added to *ex vivo* human skin tissue, protected skin from UV injury by enhancing the expression of SODs, catalase, and GPx [107]. Melatonin was further found to protect synovial mesenchymal stem cells and promote in vitro development of pronuclear embryos by enhancing gene expression of antioxidative stress genes [108, 109]. Finally, melatonin attenuated the reduction of gene expression of CuZnSOD and GPx (both cytosolic and mitochondria (GPx)) in rat liver during aging, suggesting a potentially beneficial effect of melatonin to attenuate the aging process [110].

Melatonin not only upregulates the expression of genes involved in detoxifying free radicals, it also suppresses the activity or expression of genes involved in the generation of free radicals. It is noted that the majority of studies have focused on the effect of melatonin on the genes involved in RNS production, for example, nitric oxide synthase (NOS). Bettahi et al. [111] first demonstrated that melatonin inhibited NOS activity in rat hypothalamus. Subsequent studies further confirmed this finding [55, 112]. Later, it was observed that a melatonin metabolite, AMK, also inhibited NOS activity [113]. Aside from the inhibition of the activity of NOS, melatonin was subsequently found to reduce the expression of inducible NOS (iNOS) in liver and lung of lipopolysaccharide-challenged rats [114].

It has been proposed that melatonin modulates the activities of antioxidant enzymes via its interaction with calmodulin, which in turn inhibits downstream processes that lead to the inactivation of nuclear RORα melatonin receptor [115]. Inactivation of RORα relieves the block of NF-κB-induced expression of antioxidant enzymes by reducing the expression of the upstream inhibitor of NF-κB, IκB. Therefore, the increment in antioxidant enzyme activities induced by melatonin may involve the inhibition of the RORα pathway.

Protection and improvement of mitochondrial function by melatonin

Mitochondria are a major target of melatonin [57–59, 116–119]. Both in vivo and in vitro experiments have demonstrated that melatonin protects mitochondria against oxidative damage and improves mitochondrial function (e.g. respiration and ATP production). Tan et al. [120] recently hypothesized from an evolutionary point of view that mitochondrion is the original site of melatonin synthesis in eukaryotic cells. It is therefore not difficult to imagine the evolutionarily preserved effect of melatonin on mitochondria. In addition, melatonin, as a lipophilic molecule, has been found to accumulate in high concentrations in mitochondria, suggesting that mitochondria could be a major target for melatonin [56, 60]. Finally, mitochondria are considered the main intracellular source of ROS as well as the major target of free radical damage, making it one of the most desirable targets to be protected against ROS-induced molecular mutilation.

After surveying the literature, herein we summarize the potential mechanisms or approaches by which melatonin interacts with mitochondria (Fig. 2). (i) Melatonin directly detoxifies ROS/RNS through its nonreceptor-mediated

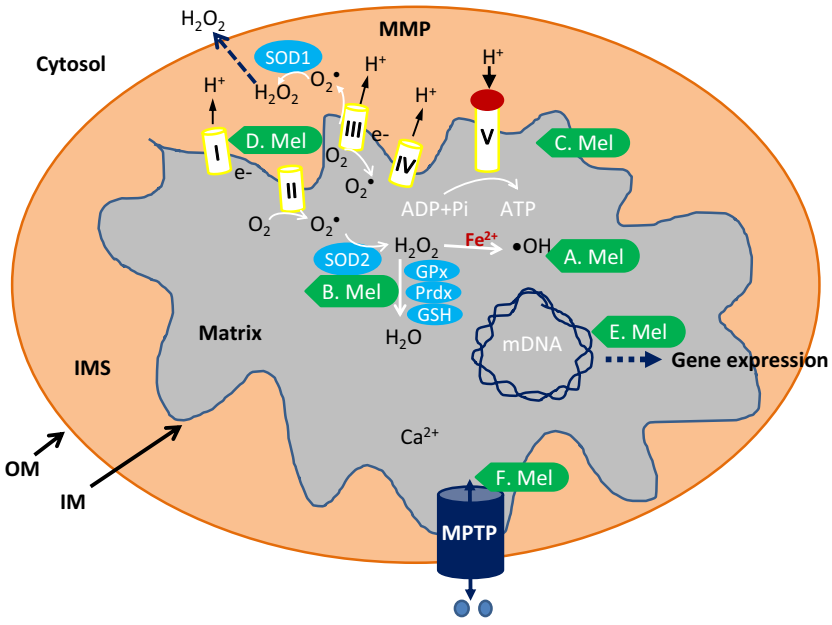

Fig. 2. Schematic representation of the potential mechanisms by which melatonin protects mitochondria. (A) Melatonin directly detoxifies ROS once it enters mitochondria. (B) Melatonin increases activities of antioxidant enzymes (SODs and GPx). (C) Melatonin stabilizes mitochondrial inner membrane. (D) Melatonin increases ETC. activity, respiration, and ATP production. (E) Melatonin regulates mitochondrial gene expression. (F) Melatonin directly regulates MPTP. Mel, melatonin; MMP, mitochondrial membrane potential; MPTP, mitochondrial permeability transition pore; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; GPx, glutathione peroxidase; Prdx, peroxiredoxin; GSH, glutathione; mDNA, mitochondrial DNA. Roman letters I, II, II, IV, and V represent mitochondrial electron transport chain complexes; IM, mitochondrial inner membrane; OM, mitochondrial outer membrane; IMS, inter membrane space.

free radical scavenging capacity, thereby lowering damage to mitochondrial proteins and DNA [121]. (ii) Melatonin increases the activity of antioxidative enzymes while suppressing pro-oxidant enzymes in mitochondria [122, 123]. (iii) Melatonin stabilizes the mitochondrial inner membrane, thereby maintaining mitochondrial integrity [124]. (iv) Melatonin increases the activity of mitochondrial electron transport chain (ETC.) complexes and improves mitochondrial respiration and ATP production, thereby reducing electron leakage and ROS generation [56]. (v) Melatonin regulates mitochondrial gene expression [121, 125]. (vi) Melatonin may directly control the currents through the mitochondrial permeability transition pore (MPTP) [126, 127]. (vii) In addition to its receptor-independent actions, melatonin modulates mitochondrial function and cell survival through MT1/MT2 receptor-mediated mechanisms [128, 129].

These mechanisms are not mutually exclusive; rather they could well be interdependent or connected. For instance, melatonin may regulate mitochondrial gene expression through MT1/MT2-dependent processes. Also, the direct ROS/RNS scavenging capacity of melatonin may help maintain mitochondrial membrane stability, ETC. activity, and MPTP. Furthermore, the lipophilic feature of melatonin allows it to accumulate in the mitochondrial membrane, thus positioning itself in the vicinity of ETC. and MPTP so that it could directly scavenge ROS generated from ETC. or interact with ETC. to facilitate electron transfer or regulate the opening of MPTP. Finally, evidence indicates that melatonin protects mitochondrial function in the brain of Alzheimer's patients through both MT1/MT2 dependent and independent mechanisms, suggesting that melatonin may preserve mitochondrial function through multiple mechanisms simultaneously [129]. Interestingly, melatonin (25–50 nM) was recently found to be effective against the parasite *Leishmania infantum* through a mechanism of disrupting the mitochondria, indicated by early MPTP opening, alteration of calcium homeostasis, increase of nitrites, and reduction of ETC. complex I, II, and III activities [130]. This exception may be due to the potentially species-specific responses to exogenous melatonin, which are in agreement with melatonin's cytotoxicity against cancer cells [131, 132].

Melatonin protects proteins of the ETC. and mitochondrial DNA from oxidative damage via its free radical scavenging capacity in vivo [133–137]. By directly detoxifying ROS/RNS, melatonin limits the loss of intra-mitochondrial glutathione, protects mitochondrial proteins and DNA from oxidative damage, and improves ETC. activity as well as ATP production. By doing so, melatonin is believed to protect against ischemia-reperfusion induced damage to the heart [138]; it also attenuates mitochondrial dysfunction in sepsis [139, 140], multiple sclerosis [141], doxorubicin-induced cellular senescence [142], in senescence-accelerated mice [143–145], and during aging [134, 146–148]. It was also found that the protection of cardiolipin from peroxidation in mitochondria was an important means by which melatonin prevents mitochondrial dysfunction [138, 146, 149–151].

In addition to its free radical scavenging capacity which protects mitochondria from oxidative damage, melatonin also exerts its protective effect on mitochondria by direct involvement in membrane integrity, permeability transition pore, electron transport, and ATP production. Garcia et al. [124] first demonstrated that melatonin helped maintain mitochondrial membrane integrity and MPTP in vitro using a microsomal system. Melatonin was further shown to interact with mitochondrial ETC. complexes I and IV to promote electron flux under basal conditions to increase ATP production [56, 152]. The high redox potential of melatonin (−0.98 V) was postulated to facilitate the electron transfer in the ETC., by donating an electron to complex I of the ETC. [57, 153]. However, the role of melatonin in mitochondrial metabolism such as oxygen consumption remains minimally investigated [154–156].

Melatonin regulates gene expression associated with mitochondria. Prunet-Marcassus et al. [125] provided the first evidence that melatonin directly affected mitochondrial gene expression. It was found that a 3 hr treatment with melatonin (10 nM to 0.1 μM) reduced mitochondrial gene expression in Siberian hamster brown adipocytes, indicated by a ~40% decrease in cytochrome b mRNA level. However, it has not been fully investigated whether this effect is achieved through direct interaction between melatonin and the mitochondrial transcriptional apparatus or through an indirect cascade of reactions initiated by melatonin. Majority of the studies indicate that melatonin modulates nuclear gene expression through both receptor-mediated and receptor-independent mechanisms, including MT1/MT2 for receptor-mediated mechanism and redox regulation (free radical scavenging) by receptor-independent mechanisms [16]. Whether melatonin modulates mitochondria-coded gene expression in a similar manner remains to be investigated.

Iñarrea et al. [123] recently found that melatonin activated mitochondrial intermembrane CuZnSOD through a mitochondrial cytochrome P450-mediated mechanism. Cytochrome P450 utilizes melatonin as a substrate to generate ROS in mitochondria (melatonin concentration ranges from 0.1 to 10 μM), thus activating CuZnSOD within the intermembrane space through oxidative modification.

Finally, melatonin was shown to inhibit the MPTP [126, 127, 157, 158]. Andrabi et al. [126] first demonstrated that melatonin directly inhibits MPTP in cultured mouse striatal neurons. Melatonin strongly inhibited MPTP currents in a dose-dependent manner with an IC50 of 0.8 μM. Consistent with its ability to inhibit MPTP, melatonin blocked N-methyl-D-aspartate (NMDA)-induced calcium rises, similar to the pattern seen with cyclosporine A (CsA), which is a blocker of MPTP. When the mouse striatal neurons were subjected to oxygen–glucose deprivation (OGD), melatonin strongly prevented the OGD-induced loss of the mitochondrial membrane potential. Thus, it was concluded that the direct inhibition of the MPTP by melatonin might essentially contribute to its anti-apoptotic effects in transient brain ischemia. Jou et al. [127, 157, 158] later also demonstrated that melatonin blocked MPTP. Using time-lapse conventional, confocal, and multiphoton fluorescent imaging microscopy coupled with noninvasive mitochondria-targeted fluorescent probes, Jou et al. [158] demonstrated that melatonin prevented

H_2O_2-induced opening of MPTP. They concluded that the prevention of MPTP opening and mitochondria membrane potential depolarization by melatonin was due to its ability to reduce mitochondrial ROS generation. Therefore, it appears that melatonin prevents the opening of the MPTP through two not mutually exclusive modes: by a direct interaction with the MPTP (shown by Andrabi's study [126]) or indirectly by suppressing upstream events that regulate the opening of the MPTP such as ROS (as shown by Jou's studies [127, 157, 158]). The later would be a result of the direct free radical scavenging capacity of melatonin.

Antioxidant capacity of melatonin's metabolites

Melatonin is metabolized in vivo through enzymatic reactions as well as chemical reactions such as oxidation to several derivatives, including 3-OHM, AFMK, AMK, 6-hydroxymelatonin sulfate, 2-hydroxymelatonin, and cyclic 3-hydroxymelatonin [9, 51, 159]). Three of these metabolites, 3-OHM, AFMK, and AMK, have been studied extensively with regard to their biological significance.

3-OHM is an immediate product of melatonin's interaction with reactive oxygen species [72–75]. 3-OHM was first detected in human and rat urine using sophisticated technologies [74, 75]. In vitro, 3-OHM was generated in a cell-free system when melatonin was incubated with H_2O_2 plus Fe^{2+}. In vivo, the level of urinary 3-OHM increased after $^{\cdot}OH$ levels were induced by ionizing radiation in rats [74]. Therefore, 3-OHM is considered a biomarker of $^{\cdot}OH$ generation. Later studies demonstrated that 3-OHM is a more potent antioxidant against $^{\cdot}OH$ and hydroperoxyl ($^{\cdot}OOH$) radicals than melatonin and its other metabolites (AMK and AFMK) [72, 73].

Mechanistically, 3-OHM functions similar to the classic antioxidants such as vitamin C, rather than to melatonin [72]. 3-OHM and classic antioxidants exhibit single phase reactions toward $ABTS^{\cdot}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), while melatonin exhibits two phases due to its cascade reactions toward radicals [72]. Using Density Functional Theory, Galano et al. [73] found that 3-OHM reacts with $^{\cdot}OH$ at diffusion-limited rates, regardless of the polarity of the environment, supporting its excellent $^{\cdot}OH$ scavenging activity. Functionally, it effectively prevents the oxidative degradation of cytochrome c induced by H_2O_2, suggesting its potential in protecting mitochondria against oxidative damage [72].

AFMK was first found to be a melatonin metabolite in rat brain by Hirata et al. [76]. In 2000, Tan et al. [77] reported for the first time that melatonin can be converted to AFMK through nonenzymatic reactions, that is, oxidation by H_2O_2. AFMK can be further oxidized to AMK by ROS/RNS [89]. Bonnefont-Rousselot et al. [160] recently demonstrated that melatonin can be oxidized to AFMK and AMK in vitro preferentially by $^{\cdot}OH$ generated by radiation, while H_2O_2 and $O_2^{\cdot-}$ did not affect the reaction. The discrepancy between these two studies could be due to the difference in experimental conditions. AFMK and AMK are the primary metabolites in vivo, which have been demonstrated in tissues, cells, and cellular compartments, including central nervous system [76], leukocytes [161], and the mitochondria [162]. Mitochondria are likely a favorite site for AFMK production due to the accumulation of melatonin in mitochondria which are the major site of ROS generation. A recent study by Niu et al. [163] raised some questions about the notion that AFMK and AMK are major melatonin metabolites. In their study, AFMK and AMK were not detected in several mouse tissues, including liver, brain, and eyes. In addition, oxidative stress induced by acetaminophen in the mouse model did not boost AFMK/AMK levels. Niu et al. [163] went further to state that AFMK/AMK formation might not be a significant pathway of melatonin disposition in mice, even under conditions of oxidative stress. However, the failure to detect AFMK and AMK may relate to their rapid metabolism to other molecules in the antioxidant cascade [116].

AFMK and AMK possess similar or even greater antioxidative capacities than melatonin [164]. AFMK has been shown to scavenge $^1O_2$ with a similar potency to melatonin [79]. AFMK reduces lipid peroxidation and oxidative DNA damage induced by a variety of oxidative stressors under various conditions [78, 165–169]. AMK has even greater efficiency than AFMK and melatonin in terms of its capacity to scavenge ROS [80, 170]. In addition to its direct antioxidant capacities, AMK reduces intracellular NO levels by inhibiting NOS activity in cytosol as well as in mitochondria [113, 171–173]. In addition, AMK, like its precursor melatonin, promotes mitochondrial complex I activity to elevate ATP production by lowering electron leakage and inhibiting opening of the mitochondrial permeability transition pore [119, 171, 172].

Aside from their antioxidative capacity, AFMK and AMK as well as melatonin exhibit anti-inflammatory and immunoregulatory activities [174, 175]. AMK was observed to inhibit the biosynthesis of prostaglandins [176]. AFMK (0.001–1 mM) inhibits tumor necrosis factor-alpha and interleukin-8 formation caused by lipopolysaccharide (LPS) in neutrophils and peripheral blood mononuclear cells. The formation of AFMK during melatonin oxidation was speculated to be an important event in the cross-talk between neutrophils and monocytes [161]. A mechanistic study has revealed that AFMK and AMK selectively inhibited gene expression of a proinflammatory enzyme, cyclooxygenase 2 (COX-2) [177]. AFMK also regulates the cell cycle of malarial parasites as does its precursor melatonin [178].

The various physiological actions of 3-OHM, AFMK, and AMK indicate that melatonin likely exerts its actions partially through its metabolites. Many other metabolites of melatonin have been identified [9]. Uncovering of the function of these metabolites will surely further expand the understanding of the function of melatonin in vivo.

Melatonin as a conditional pro-oxidant

Evidence

As discussed in early sections, the vast majority of studies have demonstrated that melatonin is a powerful scavenger for free radicals, that is, an antioxidant. However, a few

Table 1. Studies in which a pro-oxidant action of melatonin has been reported

Study	Cell type	[Melatonin]	Effect	References
In vitro	Cell free		Lipid, protein oxidation	Medina-Navarro et al. [26]
In vitro	HepG2	0.1–10 μM	Cell viability↓ (after 96 hr)	Osseni et al. [28]
		1–10 mM	GSH↓, ROS↑ (after 15 min)	
In vitro	Jurkat	0.1–1 mM	ROS↑, GSH↓, fas-induced apoptosis↑	Wölfler et al. [27]
Ex vivo	Mouse brain slice	1 mM	Redox active iron↑, heme-oxygenase-I↑	Clapp-Lilly et al. [20]
In vitro	CMK, Jurkat, MOLT-4	1 mM	Cytotoxicity↑, ROS↑	Büyükavc et al. [19]
In vitro	U937	1 mM	ROS↑, GSH↓	Albertini et al. [18]
In vitro	U937	1 mM	ROS↑, 5-LOX↑, PLA2↑, arachidonic acid↑, oxidative stress↔, viability↔	Radgona et al. [17, 21]
In vitro	U937	1 mM	NF-κB↑	Cristofannon et al. [179]
In vitro	Hematopoietic tumor cell lines	1 mM	ROS↑, viability↓, caspase activity↑	Bejarano et al. [25]
In vitro	Messangial cells	50 μM	ROS↑	Zhang et al. [23]
	Isolated mitochondria	2.5–100 μM	ROS↑	Zhang et al. [24]
In vitro	Human platelets	20–100 μM	ROS↑, intracellular calcium↑, apoptosis↑	Girish et al. [22]
In vitro	Leishmania	25–50 μM	Ca^{2+} induced mPTP opening↑, nitrites↑, mitochondria ETC.↓, parasite-toxicity↑	Elmahallawy et al. [130]

studies have reported that melatonin occasionally promotes the generation of ROS (Table 1). Medina-Navarro et al. [26] first reported that melatonin possesses pro-oxidant actions in an in vitro cell-free system, indicated by its pro-oxidant tendency toward lipids and proteins. Subsequently, other investigators observed the pro-oxidative activity of melatonin in a variety of in vitro cellular systems. Wölfler et al. [27] found that melatonin at high concentrations (10–1000 μM) was able to promote ROS generation and lead to Fas-induced apoptosis in human leukemic Jurkat cells. When tested at concentrations of <10 μM, melatonin did not induce significant ROS generation in these cancer cells. Concurrently, Clapp-Lilly et al. [20] found that melatonin at 1 mM levels increased markers of oxidative stress in an organotypic slice culture model of Alzheimer's disease, while at <100 μM, it reduced oxidative damage.

Büyükavci et al. [19] observed that high concentrations of melatonin (0.01–1 mM) induced moderate cytotoxicity with a significant increase of ROS generation in multiple human leukemia cell lines, including Jurkat cells, MOLT-4 cells, and CMK cells. The same concentration of melatonin induced elevated ROS in HL-60 leukemia cells without significant cytotoxicity, while it did not promote ROS generation or cause cytotoxicity in K562 leukemia cells and Daudi B lymphoblast cells. Bejarano et al. [25] observed that μM to mM concentrations of melatonin induced apoptosis in HL-60 cells or human platelets, associated with the activation of caspase-3 and caspase-9. In contrast, other studies reported that 1 mM melatonin, which reduced intracellular glutathione in U937 lymphoma cells, did not affect GPx activity or cell viability [18, 21]. Instead, melatonin induced a survival pathway involving NF-κB activation in U937 cells [179]. They argued that the pro-oxidant action of melatonin stimulated production of ROS which acted as secondary messengers for cell survival signaling.

In addition to cancer cells, the effects of high concentrations of melatonin on nontumor cells have also been reported. High levels of melatonin (μM to mM) did not cause cytotoxicity in several types of nontumor cells, including peripheral blood mononuclear leukocytes [21], neuronal stem cells [180], human umbilical vein endothelial cells [181], primary hepatocytes [182], and HT22, a mouse hippocampal cell line [132]. Although showing no cytotoxicity, high concentrations of melatonin did inhibit cell proliferation or arrest cell growth in some nontumor cells. However, Girish et al. [22] reported that melatonin (50 μM–1 mM) induced apoptosis in normal human platelets. These data indicate that the effects of high concentrations of melatonin are cell type dependent, that is, whether it is a cancer cell or a normal cell.

When it does occur, the induction of ROS generation by melatonin varies among different cell types. Radogna et al. [21] observed that melatonin (1 mM) induced elevated ROS as early as 1 min after its addition. However, the production of ROS was transient, which only lasted 5–6 hr. Osseni et al. [28] reported that high concentrations of melatonin (1–10 mM) induced ROS generation within 15 min after the addition of melatonin to human hepatocellular carcinoma HepG2 cells. In Jurkat cells, elevated ROS generation was measured 30 min after the addition of melatonin [27]. Other studies have observed significant rises of ROS from 2 to 48 hr after the addition of melatonin, depending on cell type [18, 19, 25]. These data indicate that the timing and duration of ROS elevation stimulated by melatonin is cell type dependent.

Interestingly, Osseni et al. [28] reported that relatively lower concentrations of melatonin (0.1–10 μM), which exhibited antioxidant action in HepG2 cells within 24 hr, became pro-oxidant after 96 hr of treatment, as indicated by the increase of glutathione within 24 hr and its depletion after 96 hr. This suggests that not only the concentration of melatonin but also the duration of the treatment with melatonin may decide the pro- versus antioxidant actions of melatonin. However, such observations have not been reported in other tumor cells or normal cells.

The evidence suggests that the pro-oxidant action of melatonin is not correlated with cytotoxicity, and it is concentration dependent as well as cell type dependent. Most importantly, the pro-oxidant action of melatonin has been observed exclusively in in vitro cell culture systems and is

mostly in cancer cells. It remains to be determined whether it occurs in vivo. As such, we may state that melatonin is a conditional pro-oxidant.

Mechanism of melatonin-mediated ROS production

The mechanism(s) by which high concentrations of melatonin stimulate ROS production remains to be determined. A recent study by Radogna et al. [21] excludes MT1/MT2 as a potential mediator for melatonin in promoting ROS generation, as indicated by the failure of a MT1/MT2-specific antagonist luzindole to block melatonin-mediated ROS generation and the failure of MT1/MT2 high-affinity melatonin analog to stimulate ROS generation. Instead, Radogna et al. [21] observed that chlorpromazine, the calmodulin-specific inhibitor, prevented not only melatonin-calmodulin interaction but also the stimulation of ROS generation by melatonin in U937 cells, suggesting that the weak interaction between calmodulin and melatonin may be involved in the stimulation of ROS by pharmacological concentrations of melatonin.

Calmodulin is a multifunctional protein, which is involved in a variety of cellular functions, including Ca^{2+} transport, protein phosphorylation, and dephosphorylation through calmodulin-dependent kinases, interaction with cAMP secondary messenger system, etc. [183]. Calmodulin binds melatonin with weak affinity [184].

Radogna et al. [17] observed that the calmodulin-mediated pro-oxidant action of melatonin involved 5-lipoxygenase (5-LOX) and phospholipase 2A (PLA2) (Fig. 3). 5-LOX catalyzes the oxygenation of arachidonic acid (AA) to form the epoxide intermediate leukotriene A_4 (LTA_4) and 5-hydroxyeicosatetraenoic acids (5-HETEs) [185]. AA usually is released from the membrane by PLA2 [186], which feeds the 5-LOX to produce LTA_4 and generate ROS. The calcium-independent PLA2 (iPLA2) can be bound and sequestered by calmodulin in an inactive form [187]. Pharmacological concentrations of melatonin freed iPLA2 from calmodulin, which released AA to feed 5-LOX for the generation of ROS in U937 cells [17]. Two PLA2-specific inhibitors, brophenacil bromide and bromoenol lactone, prevented melatonin from inducing ROS production. Addition of the 5-LOX-specific inhibitor, AA861, also abolished the ability of melatonin to stimulate ROS production, indicating the involvement of 5-LOX. An inhibitor of Ca^{2+}-dependent PLA2 (cPLA2), AACOCF3, or a cyclooxygenase-specific inhibitor, indomethacin, did not prevent melatonin from releasing AA, suggesting that cPLA2 and cyclooxygenase were not involved in this process. The same phenomenon was also observed in other cell types other than U937, including primary lymphocytes and monocytes, and human T lymphocytic Jurkat cells. Finally, a compound, chlorpromazine, which specifically interrupts the binding of melatonin to calmodulin [188], prevented melatonin-induced AA release and ROS generation; whereas another inhibitor of calmodulin, calmidazolium, which failed to prevent the binding of melatonin to calmodulin did not, suggesting the binding of melatonin to calmodulin was required.

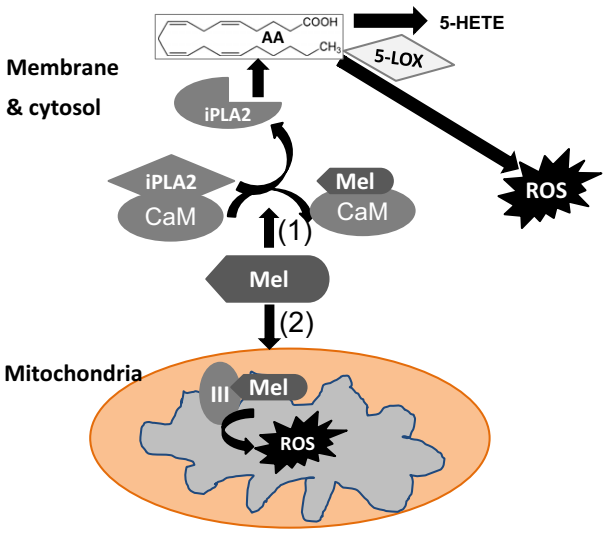

Fig. 3. Presumed mechanisms by which melatonin stimulates ROS production. Melatonin may enhance ROS production via (1) calmodulin-mediated PLA2 activation which leads to 5-LOX-mediated ROS production and (2) ETC.-mediated ROS production in mitochondria. Mel, melatonin; CaM, calmodulin; iPLA2, inducible phospholipase A2; III, mitochondrial complex III; ROS, reactive oxygen species; AA, arachidonic acid; 5-LOX, 5-lipoxygenase; 5-HETE, 5-Hydroxyeicosatetraenoic acid; III, mitochondrial complex III.

Interestingly, an earlier study reported that melatonin at a physiological concentration (1 nM) modulated the subcellular localization of calmodulin in MDCK cells [189]. Whether pharmacological levels of melatonin affect the intracellular position of calmodulin remains to be determined. One possible speculation is that different concentrations of melatonin may differentially modulate the subcellular localization of calmodulin, thereby dictating its involvement in pro-versus antioxidative activities.

Melatonin has also been reported to regulate the phosphorylation of calmodulin. Soto-Vega et al. [190] reported that melatonin stimulated the PKCα-mediated phosphorylation of calmodulin in both an in vitro reconstituted enzyme system and in cell cultures. By affecting the modification of calmodulin, melatonin can modulate the interaction between calmodulin and its targets. Whether pharmacological concentrations of melatonin affect calmodulin phosphorylation, thus ROS generation, remains to be determined.

Sarti et al. [191] recently reported that melatonin at a physiological concentration (~1 nM) transiently (within few hours) raised the expression of neuronal nNOS (transcription and translation), leading to elevated RNS (nitrite and nitrate) production in mitochondria. The increase of nNOS induced a mild reduction in oxidative phosphorylation efficiency, paralleled by a depression of the mitochondrial membrane potential and a shift to glycolysis. However, the induction of nNOS by melatonin was observed only at 1 nM but not 10 or 100 nM of melatonin. It is doubtful that pharmacological concentrations of melatonin (μM to mM) may induce nNOS expression in cytosol and mitochondria.

Although the vast majority of studies have reported that melatonin at physiological concentrations protects

mitochondria from oxidative damage, several recent studies reported that pharmacological concentrations of melatonin induced ROS generation in mitochondria isolated from rat liver cells, human mesangial cell lines, and mouse kidney [23, 24, 192]. Zhang and Zhang et al. [23, 24] observed that 1 mM of melatonin induced ROS generation through mitochondrial respiratory chain complex III in the antimycin A-sensitive site, independent of calcium. However, whether melatonin directly interacts with complex III to increase ROS production remains to be determined. Martinis et al. [192] recently reported that melatonin at 20–60 μM induced ROS generation in isolated rat liver mitochondria in the presence of pharmacological concentrations of Ca^{2+}, accompanied by a reduction of respiration and increase in the permeability transition. Iñarrea et al. [123] observed that melatonin at 0.1–10 μM induced a cytochrome P450-mediated ROS generation in isolated rat liver mitochondria. It was found that cytochrome P450 used melatonin as a substrate to generate ROS in mitochondria. Intriguingly, it was found that the production of ROS by P450 activated mitochondrial intermembrane CuZnSOD through oxidative modification, which could protect the cells from further oxidative damage. Lopez et al. [193] reported that melatonin at a wide range of concentrations (10 nM to 1 mM) protected isolated mouse liver mitochondria from oxidative damage. The discrepancy between these studies may be due to cell type specificity, experimental conditions, and measurements. Also keep in mind, results obtained from isolated mitochondria may not necessarily apply to what happens in vivo.

The site of ROS production promoted by melatonin

ROS are continuously generated as a result of normal intracellular processes in different subcellular compartments including mitochondria, peroxisomes, and cytosol (via a variety of cytosolic enzyme systems, e.g. lipoxygenases, NADPH oxidase, and cytochrome P450) [30]. Pharmacological concentrations of melatonin may promote ROS generation through its interaction with these processes in different subcellular compartments, including cytosol/membrane area and the mitochondria (Fig. 3).

Melatonin was reported to promote ROS generation through the release of AA from membrane lipid bilayers via iPLA2 in lymphocytes and monocytes, suggesting that ROS could be generated by 5-LOX adjacent to the lipid membrane where AA is released by these types of cells [17]. The demonstration of high level of accumulation of melatonin in membrane suggests that the cell membrane or its adjacent area could be a major site for melatonin to promote ROS production.

Melatonin also accumulates in mitochondria [62], which is the major site of intracellular ROS generation [193]. Mitochondria are therefore considered as another major target for melatonin, besides the lipid membrane. The identification of mitochondrial complex III as a potential site for melatonin-induced ROS production in cultured primary human mesangial cells and in mouse kidney mitochondria suggests that mitochondria respiratory chain is potentially an important site of ROS production stimulated by pharmacological concentrations of melatonin [23, 24]. However, it remains to be determined whether melatonin interacts directly with mitochondrial complex III or indirectly through other mitochondrial proteins located either in the mitochondrial matrix or the mitochondrial intermembrane space. Recent studies also observed that physiological concentrations of melatonin transiently induced the expression of nNOS in immortalized human keratinocyte cells in vitro, leading to mild decrease of oxidative phosphorylation and mitochondrial membrane potential, which could increase ROS production [191, 194].

Biological relevance

The pro-oxidant action of melatonin has been discovered in in vitro cell culture systems when melatonin is present at pharmacological concentrations (μM to mM range). It remains unknown whether the pro-oxidant action exists in vivo. It has been argued that in the tissue where melatonin is produced in vivo (e.g. the pineal gland), the concentration of melatonin could reach μM levels or even higher [9]. However, there is no evidence to date that melatonin stimulates ROS production in vivo. On the contrary, the vast majority of evidence indicates that melatonin is a potent antioxidant in vivo even at pharmacological concentrations.

The pro-oxidant action of melatonin promotes inflammatory responses and apoptosis in vitro [27, 174]. The capability of melatonin to induce apoptosis in tumor cells might have significant therapeutic implications. However, we are still far from understanding how and when melatonin becomes toxic to cancer cells but beneficial (or at least nontoxic) to healthy cells. As cancer cells are different from healthy cells in many aspects, including metabolism, gene regulation, and stress responses [195–197], cancer cells may respond to the same concentrations of melatonin differently from that of healthy cells [198].

The increased usage of exogenous melatonin as nutrient supplement alters the endogenous concentration of melatonin, which may benefit the host by reducing oxidative stress through both direct free radical scavenging capacity and molecular regulation in different tissues. In addition, the decline of melatonin production with age may render it more beneficial to supplement melatonin to the aging population to improve health by reducing free radical damage [199]. The beneficial effect of melatonin to health, disease, and aging has been supported by many in vivo animal studies. These studies have demonstrated that melatonin intake has the potential to improve cardiac function [140, 145, 200], inhibit cataract formation [201], maintain brain health [129, 146, 147], alleviate metabolic syndrome, obesity and diabetes [202, 203], reduce tumorigenesis [204, 205], protect tissues against ischemia/reperfusion injury [70, 206–212], etc. All of these beneficial effects of melatonin are associated with its antioxidant capacity.

Concluding remarks

Melatonin and its metabolites including 3-OHM, AMFK, and AFK are powerful antioxidants. They reduce

oxidative stress by several mechanisms, including direct free radical scavenging actions, gene regulation (increasing the expression of antioxidant enzymes while suppressing pro-oxidant enzymes), and/or improving mitochondrial homeostasis. The direct radical scavenging actions of melatonin are receptor-independent, while the regulation of gene expression may involve an interaction of melatonin with its conventional receptors including MT1/MT2 and possibly RZR/ROR. Melatonin and its metabolites reduce oxidative damage to proteins, lipids, and DNA. Melatonin also protects mitochondria from oxidative damage by improving or preserving mitochondrial respiration, ATP production, membrane potential and permeability transition, thus preventing electron leakage and ROS production.

While melatonin and its metabolites protect normal cells against oxidative stress-induced damage, thus preserving, protecting, or enhancing normal tissue/organism function, they have oncostatic properties in that they directly suppress cancer cell growth and/or enhance the cytotoxicity of anti-cancer drugs [213, 214]. These seemingly opposite effects of melatonin may be due to the intrinsic cellular and molecular differences between normal cells and cancer cells, obvious making these actions context specific.

The antioxidant capacity of melatonin has been well established both in vitro *and* in vivo in the last 20 yr, while its pro-oxidant property has just been observed more recently in in vitro systems. The limited data indicate that melatonin possesses pro-oxidant capacity primarily at pharmacological concentrations. However, melatonin at high concentrations does not always induce ROS/RNS. The vast majority of studies document that melatonin detoxifies ROS/RNS in μM as well as lower concentrations. In addition, the pro-oxidant action of melatonin was primarily observed in vitro in cancer cell culture systems or isolated mitochondria. Therefore, the melatonin's pro-oxidant action is context specific. Its biological relevance remains to be determined. Calmodulin, the low-affinity binding protein of melatonin, appears to mediate the pro-oxidant action of melatonin. In addition, melatonin may interact with the mitochondria to potentially promote ROS generation. A likely target is mitochondrial electron transport chain complex III.

Without doubt, the vast majority of the studies demonstrate that even pharmacological concentrations of melatonin exhibit antioxidant actions in vivo. Its beneficial effect and therapeutic value seems promising for health, aging, and various diseases including cancer, ischemia/reperfusion injury, and neurodegenerative disorders.

References

1. 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.
2. Reiter RJ, Tan DX, Rosales-Corral S et al. The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. Mini Rev Med Chem 2013; **13**:373–384.
3. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. Prog Brain Res 2010; **181**:127–151.
4. Marczynski TJ, Yamaguchi N, Ling GM et al. Sleep induced by the administration of melatonin (5-methoxyn-acetyltryptamine) to the hypothalamus in unrestrained cats. Experientia 1964; **20**:435–437.
5. Reiter RJ. Melatonin: the chemical expression of darkness. Mol Cell Endocrinol 1991; **79**:C153–C158.
6. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991; **12**:151–180.
7. Reiter RJ. The melatonin rhythm: both a clock and a calendar. Experientia 1993; **49**:654–664.
8. Reiter RJ, Tan DX, Burkhardt S. Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin. Mech Ageing Dev 2002; **123**:1007–1019.
9. Tan DX, Manchester LC, Terron MP et al. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; **42**:28–42.
10. 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.
11. Barlow-Walden LR, Reiter RJ, Abe M et al. Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int 1995; **26**:497–502.
12. Stankov B, Reiter RJ. Melatonin receptors: current status, facts, and hypotheses. Life Sci 1990; **46**:971–982.
13. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005; **27**:101–110.
14. Carrillo-Vico A, Guerrero JM, Lardone PJ et al. A review of the multiple actions of melatonin on the immune system. Endocrine 2005; **27**:189–200.
15. Tan DX, Manchester LC, Terron MP et al. Melatonin as a naturally occurring co-substrate of quinone reductase-2, the putative MT3 melatonin membrane receptor: hypothesis and significance. J Pineal Res 2007; **43**:317–320.
16. Luchetti F, Canonico B, Betti M et al. Melatonin signaling and cell protection function. FASEB J 2010; **24**:3603–3624.
17. Radogna F, Sestili P, Martinelli C et al. Lipoxygenase-mediated pro-radical effect of melatonin via stimulation of arachidonic acid metabolism. Toxicol Appl Pharmacol 2009; **238**:170–177.
18. Albertini MC, Radogna F, Accorsi A et al. Intracellular pro-oxidant activity of melatonin deprives U937 cells of reduced glutathione without affecting glutathione peroxidase activity. Ann N Y Acad Sci 2006; **1091**:10–16.
19. Buyukavci M, Ozdemir O, Buck S et al. Melatonin cytotoxicity in human leukemia cells: relation with its pro-oxidant effect. Fundam Clin Pharmacol 2006; **20**:73–79.
20. Clapp-Lilly KL, Smith MA, Perry G et al. Melatonin acts as antioxidant and pro-oxidant in an organotypic slice culture model of Alzheimer's disease. NeuroReport 2001; **12**:1277–1280.
21. Radogna F, Paternoster L, De Nicola M et al. Rapid and transient stimulation of intracellular reactive oxygen species by melatonin in normal and tumor leukocytes. Toxicol Appl Pharmacol 2009; **239**:37–45.
22. Girish KS, Paul M, Thushara RM et al. Melatonin elevates apoptosis in human platelets via ROS mediated mitochondrial damage. Biochem Biophys Res Commun 2013; **438**:198–204.

23. Zhang HM, Zhang Y, Zhang BX. The role of mitochondrial complex III in melatonin-induced ROS production in cultured mesangial cells. J Pineal Res 2011; **50**:78–82.
24. Zhang H, Zhang HM, Wu LP et al. Impaired mitochondrial complex III and melatonin responsive reactive oxygen species generation in kidney mitochondria of db/db mice. J Pineal Res 2011; **51**:338–344.
25. Bejarano I, Espino J, Barriga C et al. Pro-oxidant effect of melatonin in tumour leucocytes: relation with its cytotoxic and pro-apoptotic effects. Basic Clin Pharmacol Toxicol 2011; **108**:14–20.
26. Medina-Navarro R, Duran-Reyes G, Hicks JJ. Pro-oxidating properties of melatonin in the in vitro interaction with the singlet oxygen. Endocr Res 1999; **25**:263–280.
27. Wolfler A, Caluba HC, Abuja PM et al. Prooxidant activity of melatonin promotes fas-induced cell death in human leukemic Jurkat cells. FEBS Lett 2001; **502**:127–131.
28. Osseni RA, Rat P, Bogdan A et al. Evidence of prooxidant and antioxidant action of melatonin on human liver cell line HepG2. Life Sci 2000; **68**:387–399.
29. Valko M, Leibfritz D, Moncol J et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; **39**:44–84.
30. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; **408**:239–247.
31. Reiter RJ, Tan DX, Manchester LC et al. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. Cell Biochem Biophys 2001; **34**:237–256.
32. Patel RP, Mcandrew J, Sellak H et al. Biological aspects of reactive nitrogen species. Biochim Biophys Acta 1999; **1411**:385–400.
33. Malorni W, Pietraforte D. Focusing at the double edged sword of redox imbalance: signals for cell survival or for cell death? Antioxid Redox Signal 2014; **21**:52–55.
34. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med 2000; **29**:323–333.
35. Sinha K, Das J, Pal PB et al. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol 2013; **87**:1157–1180.
36. Lu T, Finkel T. Free radicals and senescence. Exp Cell Res 2008; **314**:1918–1922.
37. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer 1996; **32A**:30–38.
38. Moore MN. Autophagy as a second level protective process in conferring resistance to environmentally-induced oxidative stress. Autophagy 2008; **4**:254–256.
39. David SS, O'shea VL, Kundu S. Base-excision repair of oxidative DNA damage. Nature 2007; **447**:941–950.
40. Voeikov VL. Reactive oxygen species–(ROS) pathogens or sources of vital energy? Part 1. ROS in normal and pathologic physiology of living systems. J Altern Complement Med 2006; **12**:111–118.
41. Kohchi C, Inagawa H, Nishizawa T et al. ROS and innate immunity. Anticancer Res 2009; **29**:817–821.
42. Shepherd VL. The role of the respiratory burst of phagocytes in host defense. Semin Respir Infect 1986; **1**:99–106.
43. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 1991; **3**:65–70.
44. D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 2007; **8**:813–824.
45. Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med 2000; **28**:463–499.
46. Cross JV, Templeton DJ. Regulation of signal transduction through protein cysteine oxidation. Antioxid Redox Signal 2006; **8**:1819–1827.
47. Murad F. Regulation of cytosolic guanylyl cyclase by nitric oxide: the NO-cyclic GMP signal transduction system. Adv Pharmacol 1994; **26**:19–33.
48. Melchiorri D, Reiter RJ, Sewerynek E et al. Paraquat toxicity and oxidative damage. Reduction by melatonin. Biochem Pharmacol 1996; **51**:1095–1099.
49. Tan DX, Chen LD, Poeggeler B et al. Melatonin: a potent endogenous hydroxyl radical scavenger. Endocr J 1993; **1**:57–60.
50. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res 2011; **51**:1–16.
51. 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.
52. Reiter RJ, Tan DX, Manchester LC et al. Melatonin: detoxification of oxygen and nitrogen-based toxic reactants. Adv Exp Med Biol 2003; **527**:539–548.
53. Rodriguez C, Mayo JC, Sainz RM et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; **36**:1–9.
54. 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.
55. Pozo D, Reiter RJ, Calvo JR et al. Inhibition of cerebellar nitric oxide synthase and cyclic GMP production by melatonin via complex formation with calmodulin. J Cell Biochem 1997; **65**:430–442.
56. Martin M, Macias M, Escames G et al. Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J Pineal Res 2000; **28**:242–248.
57. Acuna-Castroviejo D, Martin M, Macias M et al. Melatonin, mitochondria, and cellular bioenergetics. J Pineal Res 2001; **30**:65–74.
58. Leon J, Acuna-Castroviejo D, Sainz RM et al. Melatonin and mitochondrial function. Life Sci 2004; **75**:765–790.
59. Acuna-Castroviejo D, Lopez LC, Escames G et al. Melatonin-mitochondria interplay in health and disease. Curr Top Med Chem 2011; **11**:221–240.
60. Menendez-Pelaez A, Reiter RJ. Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. J Pineal Res 1993; **15**:59–69.
61. Menendez-Pelaez A, Poeggeler B, Reiter RJ et al. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 1993; **53**:373–382.
62. Venegas C, Garcia JA, Escames G et al. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J Pineal Res 2012; **52**:217–227.
63. Poeggeler B, Saarela S, Reiter RJ et al. Melatonin–a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole accessed in vitro. Ann N Y Acad Sci 1994; **738**:419–420.

64. 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.
65. Susa N, Ueno S, Furukawa Y et al. Potent protective effect of melatonin on chromium(VI)-induced DNA single-strand breaks, cytotoxicity, and lipid peroxidation in primary cultures of rat hepatocytes. Toxicol Appl Pharmacol 1997; **144**:377–384.
66. Stasica P, Ulanski P, Rosiak JM. Melatonin as a hydroxyl radical scavenger. J Pineal Res 1998; **25**:65–66.
67. Roberts JE, Hu DN, Wishart JF. Pulse radiolysis studies of melatonin and chloromelatonin. J Photochem Photobiol B 1998; **42**:125–132.
68. Rath MC, Mahal HS, Mukherjee T. Photophysics of melatonin in different environments. Photochem Photobiol 1999; **69**:294–300.
69. Pahkla R, Zilmer M, Kullisaar T et al. Comparison of the antioxidant activity of melatonin and pinoline in vitro. J Pineal Res 1998; **24**:96–101.
70. Li XJ, Zhang LM, Gu J et al. Melatonin decreases production of hydroxyl radical during cerebral ischemia-reperfusion. Zhongguo Yao Li Xue Bao 1997; **18**:394–396.
71. Bromme HJ, Morke W, Peschke D et al. Scavenging effect of melatonin on hydroxyl radicals generated by alloxan. J Pineal Res 2000; **29**:201–208.
72. Tan DX, Hardeland R, Manchester LC et al. Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicals and suppresses oxidative reactions. Curr Med Chem 2014; **21**:1557–1565.
73. Galano A, Tan DX, Reiter RJ. Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. Roy Soc Chem Adv 2014; **4**:5220–5227.
74. 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.
75. Tan DX, Manchester LC, Reiter RJ et al. Cyclic 3-hydroxymelatonin: a melatonin metabolite generated as a result of hydroxyl radical scavenging. Biol Signals Recept 1999; **8**:70–74.
76. Hirata F, Hayaishi O, Tokuyama T et al. In vitro and in vivo formation of two new metabolites of melatonin. J Biol Chem 1974; **249**:1311–1313.
77. Tan DX, Manchester LC, Reiter RJ et al. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. Free Radic Biol Med 2000; **29**:1177–1185.
78. Tan DX, Manchester LC, Burkhardt S et al. N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. FASEB J 2001; **15**:2294–2296.
79. Maharaj DS, Anoopkumar-Dukie S, Glass BD et al. The identification of the UV degradants of melatonin and their ability to scavenge free radicals. J Pineal Res 2002; **32**:257–261.
80. 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.
81. Guenther AL, Schmidt SI, Laatsch H et al. Reactions of the melatonin metabolite AMK (N1-acetyl-5-methoxykynuramine) with reactive nitrogen species: formation of novel compounds, 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. J Pineal Res 2005; **39**:251–260.
82. 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**:181–197.
83. Reiter RJ, Paredes SD, Manchester LC et al. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol 2009; **44**:175–200.
84. Paredes SD, Reiter RJ. Melatonin: helping cells cope with oxidative disaster. Cell Membr Free Radic Res 2010; **2**:99–111.
85. Mahal HS, Sharma HS, Mukherjee T. Antioxidant properties of melatonin: a pulse radiolysis study. Free Radic Biol Med 1999; **26**:557–565.
86. Scaiano JC. Exploratory laser flash photolysis study of free radical reactions and magnetic field effects in melatonin chemistry. J Pineal Res 1995; **19**:189–195.
87. Zhang H, Squadrito GL, Pryor WA. The reaction of melatonin with peroxynitrite: formation of melatonin radical cation and absence of stable nitrated products. Biochem Biophys Res Commun 1998; **251**:83–87.
88. Zhang H, Squadrito GL, Uppu R et al. Reaction of peroxynitrite with melatonin: a mechanistic study. Chem Res Toxicol 1999; **12**:526–534.
89. Zang LY, Cosma G, Gardner H et al. Scavenging of reactive oxygen species by melatonin. Biochim Biophys Acta 1998; **1425**:469–477.
90. Poeggeler B, Reiter RJ, Tan DX et al. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis. J Pineal Res 1993; **14**:151–168.
91. Cagnoli CM, Atabay C, Kharlamova E et al. Melatonin protects neurons from singlet oxygen-induced apoptosis. J Pineal Res 1995; **18**:222–226.
92. Noda Y, Mori A, Liburdy R et al. Melatonin and its precursors scavenge nitric oxide. J Pineal Res 1999; **27**:159–163.
93. Blanchard B, Pompon D, Ducrocq C. Nitrosation of melatonin by nitric oxide and peroxynitrite. J Pineal Res 2000; **29**:184–192.
94. Gilad E, Cuzzocrea S, Zingarelli B et al. Melatonin is a scavenger of peroxynitrite. Life Sci 1997; **60**:PL169–PL174.
95. Mayo JC, Tan DX, Sainz RM et al. Oxidative damage to catalase induced by peroxyl radicals: functional protection by melatonin and other antioxidants. Free Radic Res 2003; **37**:543–553.
96. Cuzzocrea S, Zingarelli B, Costantino G et al. Protective effect of melatonin in a non-septic shock model induced by zymosan in the rat. J Pineal Res 1998; **25**:24–33.
97. Cuzzocrea S, Costantino G, Caputi AP. Protective effect of melatonin on cellular energy depletion mediated by peroxynitrite and poly (ADP-ribose) synthetase activation in a non-septic shock model induced by zymosan in the rat. J Pineal Res 1998; **25**:78–85.
98. El-Sokkary GH, Reiter RJ, Cuzzocrea S et al. Role of melatonin in reduction of lipid peroxidation and peroxynitrite formation in non-septic shock induced by zymosan. Shock 1999; **12**:402–408.
99. Dugo L, Serraino I, Fulia F et al. Effect of melatonin on cellular energy depletion mediated by peroxynitrite and poly (ADP-ribose) synthetase activation in an acute model of inflammation. J Pineal Res 2001; **31**:76–84.

100. Yin J, Liu YH, Xu YF et al. Melatonin arrests peroxynitrite-induced tau hyperphosphorylation and the overactivation of protein kinases in rat brain. J Pineal Res 2006; **41**:124–129.
101. Antolin I, Rodriguez C, Sainz RM et al. Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. FASEB J 1996; **10**:882–890.
102. Mayo JC, Sainz RM, Antoli I et al. Melatonin regulation of antioxidant enzyme gene expression. Cell Mol Life Sci 2002; **59**:1706–1713.
103. Tang YP, Ma YL, Chao CC et al. Enhanced glial cell line-derived neurotrophic factor mRNA expression upon (-)-deprenyl and melatonin treatments. J Neurosci Res 1998; **53**:593–604.
104. Gomez M, Esparza JL, Nogues MR et al. Pro-oxidant activity of aluminum in the rat hippocampus: gene expression of antioxidant enzymes after melatonin administration. Free Radic Biol Med 2005; **38**:104–111.
105. Esparza JL, Gomez M, Rosa Nogues M et al. Melatonin reduces oxidative stress and increases gene expression in the cerebral cortex and cerebellum of aluminum-exposed rats. J Pineal Res 2005; **39**:129–136.
106. Kotler M, Rodriguez C, Sainz RM et al. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res 1998; **24**:83–89.
107. Fischer TW, Kleszczynski K, Hardkop LH et al. Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVR-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.
108. Wang F, Tian X, Zhang L et al. Melatonin promotes the in vitro development of pronuclear embryos and increases the efficiency of blastocyst implantation in murine. J Pineal Res 2013; **55**:267–274.
109. Liu X, Xu Y, Chen S et al. Rescue of proinflammatory cytokine-inhibited chondrogenesis by the antiarthritic effect of melatonin in synovium mesenchymal stem cells via suppression of reactive oxygen species and matrix metalloproteinases. Free Radic Biol Med 2014; **68**:234–246.
110. Mauriz JL, Molpeceres V, Garcia-Mediavilla MV et al. Melatonin prevents oxidative stress and changes in antioxidant enzyme expression and activity in the liver of aging rats. J Pineal Res 2007; **42**:222–230.
111. Bettahi I, Pozo D, Osuna C et al. Melatonin reduces nitric oxide synthase activity in rat hypothalamus. J Pineal Res 1996; **20**:205–210.
112. Leon J, Vives F, Crespo E et al. Modification of nitric oxide synthase activity and neuronal response in rat striatum by melatonin and kynurenine derivatives. J Neuroendocrinol 1998; **10**:297–302.
113. Leon J, Escames G, Rodriguez MI et al. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. J Neurochem 2006; **98**:2023–2933.
114. Crespo E, Macias M, Pozo D et al. Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. FASEB J 1999; **13**:1537–1546.
115. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res 2005; **39**:99–104.
116. Reiter RJ, Paredes SD, Korkmaz A et al. Melatonin combats molecular terrorism at the mitochondrial level. Interdiscip Toxicol 2008; **1**:137–149.
117. Acuna-Castroviejo D, Escames G, Rodriguez MI et al. Melatonin role in the mitochondrial function. Front Biosci 2007; **12**:947–963.
118. Leon J, Acuna-Castroviejo D, Escames G et al. Melatonin mitigates mitochondrial malfunction. J Pineal Res 2005; **38**:1–9.
119. Acuna-Castroviejo D, Escames G, Leon J et al. Mitochondrial regulation by melatonin and its metabolites. Adv Exp Med Biol 2003; **527**:549–557.
120. 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 evolution in eukaryotes. J Pineal Res 2013; **54**:127–238.
121. Acuna-Castroviejo D, Escames G, Carazo A et al. Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. Curr Top Med Chem 2002; **2**:133–151.
122. Acuna-Castroviejo D, Escames G, Lopez LC et al. Melatonin and nitric oxide: two required antagonists for mitochondrial homeostasis. Endocrine 2005; **27**:159–168.
123. Inarrea P, Casanova A, Alava MA et al. Melatonin and steroid hormones activate intermembrane Cu,Zn-superoxide dismutase by means of mitochondrial cytochrome P450. Free Radic Biol Med 2011; **50**:1575–1581.
124. Garcia JJ, Reiter RJ, Pie J et al. Role of pinoline and melatonin in stabilizing hepatic microsomal membranes against oxidative stress. J Bioenerg Biomembr 1999; **31**:609–616.
125. Prunet-Marcassus B, Ambid L, Viguerie-Bascands N et al. Evidence for a direct effect of melatonin on mitochondrial genome expression of Siberian hamster brown adipocytes. J Pineal Res 2001; **30**:108–115.
126. Andrabi SA, Sayeed I, Siemen D et al. Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. FASEB J 2004; **18**:869–871.
127. Jou MJ. Melatonin preserves the transient mitochondrial permeability transition for protection during mitochondrial Ca^{2+} stress in astrocyte. J Pineal Res 2011; **50**:427–435.
128. Radogna F, Cristofanon S, Paternoster L et al. Melatonin antagonizes the intrinsic pathway of apoptosis via mitochondrial targeting of Bcl-2. J Pineal Res 2008; **44**:316–325.
129. Dragicevic N, Copes N, O'Neal-Moffitt G et al. Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. J Pineal Res 2011; **51**:75–86.
130. Elmahallawy EK, Jimenez-Aranda A, Martinez AS et al. Activity of melatonin against *Leishmania infantum* promastigotes by mitochondrial dependent pathway. Chem Biol Interact 2014; **220**:84–93.
131. 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.
132. Rodriguez C, Martin V, Herrera F et al. Mechanisms involved in the pro-apoptotic effect of melatonin in cancer cells. Int J Mol Sci 2013; **14**:6597–6613.
133. Karbownik M, Tan D, Manchester LC et al. Renal toxicity of the carcinogen delta-aminolevulinic acid: antioxidant effects of melatonin. Cancer Lett 2000; **161**:1–7.
134. Reiter RJ, Tan DX, Manchester LC et al. Melatonin reduces oxidant damage and promotes mitochondrial

respiration: implications for aging. Ann N Y Acad Sci 2002; **959**:238–250.
135. Yamamoto HA, Mohanan PV. Melatonin attenuates brain mitochondria DNA damage induced by potassium cyanide in vivo and in vitro. Toxicology 2002; **179**:29–36.
136. Chen LJ, Gao YQ, Li XJ et al. Melatonin protects against MPTP/MPP+ -induced mitochondrial DNA oxidative damage in vivo and in vitro. J Pineal Res 2005; **39**:34–42.
137. Guha M, Maity P, Choubey V et al. Melatonin inhibits free radical-mediated mitochondrial-dependent hepatocyte apoptosis and liver damage induced during malarial infection. J Pineal Res 2007; **43**:372–381.
138. Petrosillo G, Di Venosa N, Pistolese M et al. Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia- reperfusion: role of cardiolipin. FASEB J 2006; **20**:269–276.
139. Lopez LC, Escames G, Ortiz F et al. Melatonin restores the mitochondrial production of ATP in septic mice. Neuro Endocrinol Lett 2006; **27**:623–630.
140. Zhang H, Liu D, Wang X et al. Melatonin improved rat cardiac mitochondria and survival rate in septic heart injury. J Pineal Res 2013; **55**:1–6.
141. Kashani IR, Rajabi Z, Akbari M et al. Protective effects of melatonin against mitochondrial injury in a mouse model of multiple sclerosis. Exp Brain Res 2014 [Epub ahead of print].
142. Song N, Kim AJ, Kim HJ et al. Melatonin suppresses doxorubicin-induced premature senescence of A549 lung cancer cells by ameliorating mitochondrial dysfunction. J Pineal Res 2012; **53**:335–343.
143. Rodriguez MI, Escames G, Lopez LC et al. Improved mitochondrial function and increased life span after chronic melatonin treatment in senescent prone mice. Exp Gerontol 2008; **43**:749–756.
144. Okatani Y, Wakatsuki A, Reiter RJ. Melatonin protects hepatic mitochondrial respiratory chain activity in senescence-accelerated mice. J Pineal Res 2002; **32**:143–148.
145. Rodriguez MI, Carretero M, Escames G et al. Chronic melatonin treatment prevents age-dependent cardiac mitochondrial dysfunction in senescence-accelerated mice. Free Radic Res 2007; **41**:15–24.
146. Petrosillo G, Fattoretti P, Matera M et al. Melatonin prevents age-related mitochondrial dysfunction in rat brain via cardiolipin protection. Rejuvenation Res 2008; **11**:935–943.
147. Carretero M, Escames G, Lopez LC et al. Long-term melatonin administration protects brain mitochondria from aging. J Pineal Res 2009; **47**:192–200.
148. Acuna-Castroviejo D, Carretero M, Doerrier C et al. Melatonin protects lung mitochondria from aging. Age 2012; **34**:681–692.
149. Petrosillo G, Moro N, Ruggiero FM et al. Melatonin inhibits cardiolipin peroxidation in mitochondria and prevents the mitochondrial permeability transition and cytochrome c release. Free Radic Biol Med 2009; **47**:969–974.
150. Petrosillo G, De Benedictis V, Ruggiero FM et al. Decline in cytochrome c oxidase activity in rat-brain mitochondria with aging. Role of peroxidized cardiolipin and beneficial effect of melatonin. J Bioenerg Biomembr 2013; **45**:431–440.
151. Luchetti F, Canonico B, Mannello F et al. Melatonin reduces early changes in intramitochondrial cardiolipin during apoptosis in U937 cell line. Toxicol In Vitro 2007; **21**:293–301.
152. Martin M, Macias M, Leon J et al. Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. Int J Biochem Cell Biol 2002; **34**:348–357.
153. Reiter RJ. Melatonin: lowering the high price of free radicals. News Physiol Sci 2000; **15**:246–250.
154. De Atenor MS, De Romero IR, Brauckmann E et al. Effects of the pineal gland and melatonin on the metabolism of oocytes in vitro and on ovulation in *Bufo arenarum*. J Exp Zool 1994; **268**:436–441.
155. Reyes-Toso CF, Ricci CR, De Mignone IR et al. In vitro effect of melatonin on oxygen consumption in liver mitochondria of rats. Neuro Endocrinol Lett 2003; **24**:341–344.
156. Reyes-Toso CF, Rebagliati IR, Ricci CR et al. Effect of melatonin treatment on oxygen consumption by rat liver mitochondria. Amino Acids 2006; **31**:299–302.
157. Jou MJ, Peng TI, Yu PZ et al. Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. J Pineal Res 2007; **43**:389–403.
158. Jou MJ, Peng TI, Reiter RJ et al. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. J Pineal Res 2004; **37**:55–70.
159. Reiter RJ, Tan DX, Terron MP et al. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. Acta Biochim Pol 2007; **54**:1–9.
160. Bonnefont-Rousselot D, Collin F, Jore D et al. Reaction mechanism of melatonin oxidation by reactive oxygen species in vitro. J Pineal Res 2011; **50**:328–335.
161. Silva SO, Rodrigues MR, Carvalho SR et al. Oxidation of melatonin and its catabolites, N1-acetyl-N2 -formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine, by activated leukocytes. J Pineal Res 2004; **37**:171–175.
162. Semak I, Naumova M, Korik E et al. A novel metabolic pathway of melatonin: oxidation by cytochrome C. Biochemistry 2005; **44**:9300–9307.
163. Niu S, Li F, Tan DX et al. Analysis of N1-acetyl-N2-formyl-5-methoxykynuramine/N1-acetyl-5-methoxy-kynuramine formation from melatonin in mice. J Pineal Res 2010; **49**:106–114.
164. 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.
165. Liu X, Chen Z, Chua CC et al. Melatonin as an effective protector against doxorubicin-induced cardiotoxicity. Am J Physiol Heart Circ Physiol 2002; **283**:H254–H263.
166. Burkhardt S, Reiter RJ, Tan DX et al. DNA oxidatively damaged by chromium(III) and H(2)O(2) is protected by the antioxidants melatonin, N(1)-acetyl-N(2)-formyl-5-methoxykynuramine, resveratrol and uric acid. Int J Biochem Cell Biol 2001; **33**:775–783.
167. Onuki J, Almeida EA, Medeiros MH et al. Inhibition of 5-aminolevulinic acid-induced DNA damage by melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, quercetin or resveratrol. J Pineal Res 2005; **38**:107–115.
168. Janjetovic Z, Nahmias ZP, Hanna S et al. Melatonin and its metabolites ameliorate UVB-induced damages in human epidermal keratinocytes. J Pineal Res 2014; **57**:90–102.
169. Manda K, Ueno M, Anzai K. AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. J Pineal Res 2007; **42**:386–393.

170. Schaefer M, Hardeland R. The melatonin metabolite N-acetyl-5-methoxykynuramine is a potent singlet oxygen scavenger. J Pineal Res 2009; **46**:49–52.
171. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 2005; **27**:119–130.
172. Tapias V, Escames G, Lopez LC et al. Melatonin and its brain metabolite N(1)-acetyl-5-methoxykynuramine prevent mitochondrial nitric oxide synthase induction in parkinsonian mice. J Neurosci Res 2009; **87**:3002–3010.
173. Entrena A, Camacho ME, Carrion MD et al. Kynuramines as neural nitric oxide synthase inhibitors. J Med Chem 2005; **48**:8174–8181.
174. Radogna F, Diederich M, Ghibelli L. Melatonin: a pleiotropic molecule regulating inflammation. Biochem Pharmacol 2010; **80**:1844–1852.
175. 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**:1–14.
176. Kelly RW, Amato F, Seamark RF. N-acetyl-5-methoxy kynurenamine, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin biosynthesis. Biochem Biophys Res Commun 1984; **121**:372–379.
177. Mayo JC, Sainz RM, Tan DX et al. Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. J Neuroimmunol 2005; **165**:139–149.
178. Budu A, Peres R, Bueno VB et al. N1-acetyl-N2-formyl-5-methoxykynuramine modulates the cell cycle of malaria parasites. J Pineal Res 2007; **42**:261–266.
179. Cristofanon S, Uguccioni F, Cerella C et al. Intracellular prooxidant activity of melatonin induces a survival pathway involving NF-kappaB activation. Ann N Y Acad Sci 2009; **1171**:472–478.
180. Fu J, Zhao SD, Liu HJ et al. Melatonin promotes proliferation and differentiation of neural stem cells subjected to hypoxia in vitro. J Pineal Res 2011; **51**:104–112.
181. Cui P, Yu M, Peng X et al. Melatonin prevents human pancreatic carcinoma cell PANC-1-induced human umbilical vein endothelial cell proliferation and migration by inhibiting vascular endothelial growth factor expression. J Pineal Res 2012; **52**:236–243.
182. Kojima T, Mochizuki C, Mitaka T et al. Effects of melatonin on proliferation, oxidative stress and Cx32 gap junction protein expression in primary cultures of adult rat hepatocytes. Cell Struct Funct 1997; **22**:347–356.
183. James P, Vorherr T, Carafoli E. Calmodulin-binding domains: just two faced or multi-faceted? Trends Biochem Sci 1995; **20**:38–42.
184. Benitez-King G, Huerto-Delgadillo L, Anton-Tay F. Binding of 3H-melatonin to calmodulin. Life Sci 1993; **53**:201–207.
185. Ford-Hutchinson AW, Gresser M, Young RN. 5-Lipoxygenase. Annu Rev Biochem 1994; **63**:383–417.
186. Dennis EA, Cao J, Hsu YH et al. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev 2011; **111**:6130–6185.
187. Jenkins CM, Wolf MJ, Mancuso DJ et al. Identification of the calmodulin-binding domain of recombinant calcium-independent phospholipase A2beta. implications for structure and function. J Biol Chem 2001; **276**:7129–7135.
188. Romero MP, Garcia-Perganeda A, Guerrero JM et al. Membrane-bound calmodulin in Xenopus laevis oocytes as a novel binding site for melatonin. FASEB J 1998; **12**:1401–1408.
189. Anton-Tay F, Martinez I, Tovar R et al. Modulation of the subcellular distribution of calmodulin by melatonin in MDCK cells. J Pineal Res 1998; **24**:35–42.
190. Soto-Vega E, Meza I, Ramirez-Rodriguez G et al. Melatonin stimulates calmodulin phosphorylation by protein kinase C. J Pineal Res 2004; **37**:98–106.
191. Sarti P, Magnifico MC, Altieri F et al. New evidence for cross talk between melatonin and mitochondria mediated by a circadian-compatible interaction with nitric oxide. Int J Mol Sci 2013; **14**:11259–11276.
192. Martinis P, Zago L, Maritati M et al. Interactions of melatonin with mammalian mitochondria. Reducer of energy capacity and amplifier of permeability transition. Amino Acids 2012; **42**:1827–1837.
193. Lopez A, Garcia JA, Escames G et al. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. J Pineal Res 2009; **46**:188–198.
194. Arese M, Magnifico MC, Mastronicola D et al. Nanomolar melatonin enhances nNOS expression and controls HaCaT-cells bioenergetics. IUBMB Life 2012; **64**:251–258.
195. Kabore AF, Johnston JB, Gibson SB. Changes in the apoptotic and survival signaling in cancer cells and their potential therapeutic implications. Curr Cancer Drug Targets 2004; **4**:147–163.
196. Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. Drug Resist Updat 2004; **7**:97–110.
197. Gogvadze V, Zhivotovsky B, Orrenius S. The Warburg effect and mitochondrial stability in cancer cells. Mol Aspects Med 2010; **31**:60–74.
198. Sainz RM, Mayo JC, Rodriguez C et al. Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. Cell Mol Life Sci 2003; **60**:1407–1426.
199. Karasek M, Reiter RJ. Melatonin and aging. Neuro Endocrinol Lett 2002; **23**(Suppl. 1):14–16.
200. Teshima Y, Takahashi N, Nishio S et al. Production of reactive oxygen species in the diabetic heart. Roles of mitochondria and NADPH oxidase. Circ J 2014; **78**:300–306.
201. 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.
202. Cheshchevik VT, Dremza IK, Lapshina EA et al. Corrections by melatonin of liver mitochondrial disorders under diabetes and acute intoxication in rats. Cell Biochem Funct 2011; **29**:481–488.
203. Reiter RJ, Tan DX, Korkmaz A et al. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. Ann Med 2012; **44**:564–577.
204. Sharman EH, Sharman KG, Bondy SC. Extended exposure to dietary melatonin reduces tumor number and size in aged male mice. Exp Gerontol 2011; **46**:18–22.
205. Kim KJ, Choi JS, Kang I et al. Melatonin suppresses tumor progression by reducing angiogenesis stimulated by

HIF-1 in a mouse tumor model. J Pineal Res 2013; **54**:264–270.
206. Okatani Y, Wakatsuki A, Reiter RJ et al. Protective effect of melatonin against mitochondrial injury induced by ischemia and reperfusion of rat liver. Eur J Pharmacol 2003; **469**:145–152.
207. Watanabe K, Wakatsuki A, Shinohara K et al. Maternally administered melatonin protects against ischemia and reperfusion-induced oxidative mitochondrial damage in premature fetal rat brain. J Pineal Res 2004; **37**:276–280.
208. Dominguez-Rodriguez A, Arroyo-Ucar E, Abreu-Gonzalez P. Role of melatonin in preventing mitochondrial dysfunction in myocardial ischemia-reperfusion injury. Am J Cardiol 2010; **106**:1521–1522.
209. 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.
210. Giacomo CG, Antonio M. Melatonin in cardiac ischemia/reperfusion-induced mitochondrial adaptive changes. Cardiovasc Hematol Disord Drug Targets 2007; **7**:163–169.
211. Reiter RJ, Tan DX, Leon J et al. When melatonin gets on your nerves: its beneficial actions in experimental models of stroke. Exp Biol Med 2005; **230**:104–117.
212. 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.
213. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX et al. Basic mechanisms involved in the anti-cancer effects of melatonin. Curr Med Chem 2010; **17**:4462–4481.
214. 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.