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Prooxidant effects of melatonin: a brief review

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Abstract: Melatonin acts classically through the widely expressed G protein-coupled membrane receptors MT_1 and MT_2, respectively. The functional role of the MT receptors is not fully clear with multiple effects, such as effects on the circadian, reproductive, immune, cardiovascular, and intestinal systems, being suggested. In addition to receptor-mediated effects, melatonin also acts as a potent antioxidant and it is quite evident that melatonin and its metabolites efficiently reduce oxidative damage to proteins, lipids, and DNA and also exert protective effects on mitochondria. The potent antioxidant activity of melatonin stems from various complex mechanisms, including direct scavenging of radicals, stimulation of antioxidant enzymes, and maintenance of mitochondrial homeostasis. However, about a dozen experimental studies also suggest that melatonin can exert prooxidant effects under certain circumstances. Involvement of calmodulin and the mitochondrial respiratory chain may be potential targets for the prooxidant effects of melatonin. This review briefly summarizes the physiobiochemistry of melatonin, including its antioxidative functions, and summarizes and discusses studies showing prooxidant effects of this hormone.

Key words: Melatonin, physiology, free radicals, mitochondria, antioxidative effects, prooxidant effects

1. Introduction

Melatonin, chemically N-acetyl-5-methoxytryptamine, was primarily isolated from bovine pineal glands and its structure was first identified in 1958 (Lerner et al., 1958). This indolamine was later discovered to be also present or synthesized in extrapineal tissues such as the retina, gastrointestinal tract, Harderian gland, testes, and lymphocytes (Reiter et al., 2013). Due to the fact that melatonin possesses hydrophilic and lipophilic characteristics, its lipophilic ability enables it to penetrate all biological membranes, including the blood–brain barrier (Reiter et al., 1997). Generally, melatonin derives from the essential amino acid tryptophan by a multistep enzymatic reaction chain, including tryptophan 5-hydroxylation, decarboxylation, N-acetylation, and O-methylation. Furthermore, melatonin can be synthesized via O-methylation of serotonin and subsequent N-acetylation of 5-methoxytryptamine, or by O-methylation of tryptophan, followed by decarboxylation and N-acetylation (Hardeland et al., 1993). From a functional perspective, melatonin is characterized as a hormone involved in the regulation of the circadian rhythm of several biological functions and it also plays an important role in immunoregulation, reproduction, sleep, and inflammatory responses (Hardeland and Fuhrberg, 1996; Reiter et al., 2000). MT_1 and MT_2 are two well-characterized G protein-coupled plasma membrane melatonin receptors, which are activated by melatonin and regulate multiple cellular and physiological functions, including neuronal activity, arterial vasoconstriction, cell proliferation, immune responses, reproduction, and metabolic functions (Stankov and Reiter, 1990; Dubocovich and Markowska, 2005; Ekmekçioğlu, 2006, 2014). In addition, melatonin has high-affinity binding for nuclear receptors ROR/RZR, which operate as transcriptional activators, and it also interacts with other intracellular proteins, like quinone reductase-2 (or MT_3) and calmodulin (Reppert et al., 1994; Wiesenberg et al., 1998). For the regulation of gene expression, it has been proposed that ROR/RZR work in cooperation with the plasma membrane receptors MT_1/MT_2 (Carrillo-Vico et al., 2005). Regarding the regulation of the cellular redox status, melatonin possibly interacts with quinone reductase, even though the detailed role of this interaction remains poorly understood (Tan et al., 2007).

Melatonin and calmodulin possess low-affinity interaction, which may relate to their antioxidant actions, as well as other signaling processes (Luchetti et al., 2010). Various studies describe melatonin and its derivatives as broad-spectrum antioxidants, related to their ability to

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act as potent free radical scavengers (Reiter et al., 2002; Tan et al., 2007). While the regulation of gene expression may imply an interaction of melatonin with its common receptors MT_1/MT_2 and possibly also RZR/ROR, the direct radical scavenging actions of melatonin are usually receptor-independent. In general, it is well established that melatonin and its metabolites reduce oxidative damage to proteins, lipids, and DNA. They also play a protective role in mitochondria, by preventing them from undergoing oxidative damage. Therefore, melatonin improves or preserves ATP production, mitochondrial respiration, membrane potential, and permeability transition and consequently prevents electron leakage and reactive oxygen species (ROS) production (Zhang and Zhang, 2014). In addition, studies from the last years suggested that melatonin also exerts prooxidant effects under certain circumstances (Zhang and Zhang, 2014). This brief review will especially focus on this topic.

2. Reactive oxygen species and oxidative stress

ROS result as natural byproducts of the normal metabolism of oxygen and are considered as highly reactive molecules because of their presence of unpaired electrons (Rezaie et al., 2007). In this regard free radicals are small, diffusible, and reactive molecules that participate in chain reactions, in which even a single free radical initiation event could distribute damage to multiple molecules (Jones, 2008).

Vital tissues consist of complex antioxidative defense systems to avoid the destructive effects implicated by ROS (Table 1). A high ROS burden leads to oxidative stress, which is defined as a marked imbalance between the production of ROS and their elimination by antioxidants (Rezaie et al., 2007). In general, macromolecular damage is typically observed in the broader sense of oxidative mechanisms, linked to free radicals (Jones, 2008).

The toxic species that could be responsible for diseases and aging are in general categorized in two groups: ROS and reactive nitrogen species (RNS). Singlet oxygen ($O_2^{\bullet}$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$) belong to ROS (Halliwell, 2006), whereby the hydroxyl radical is responsible for the largest amount of damage in living cells (Finkel and Holbrook, 2000). On the other hand, nitric oxide and especially its product peroxynitrite are included in the RNS category (Packer et al., 1996).

Free radicals are produced during normal mitochondrial metabolism and also by a variety of cytosolic enzyme systems, like lipoxygenase, NADPH-oxidase, and cytochrome P450 (Finkel and Holbrook, 2000). The respiratory chain is, so far as we know, a powerful source of ROS, and there are two major regions where ROS are produced, one being complex I (NADPH coenzyme Q reductase) and the other complex III (ubiquinol cytochrome c reductase) (Fridovich, 1986; Cross and Jones, 1991). Due to the exposure to highly concentrated ROS, mitochondrial structures are particularly predisposed to free radical attack (Valls et al., 1994). Here, lipid peroxidation, protein oxidation, and mitochondrial DNA mutations can be consequences of the oxidative damage to mitochondrial components (Richter et al., 1988; Stadtman, 1992; Ernster, 1993).

Furthermore, free radicals can have deleterious consequences at the cellular level, like cell death or tumor genesis. Free radicals enhance activation of caspases, cell cycle control protein p53, cytochrome c release from mitochondria, and other apoptotic signaling proteins

Table 1. Major endo- and exogenous antioxidants.

Endogenous antioxidants	Exogenous antioxidants
Enzymatic antioxidants: -Superoxide dismutase (SOD) – dependent on manganese, zinc, and copper -Catalase (CAT) – dependent on iron -Glutathione peroxidase (GPx) – dependent on selenium -Glutathione reductase (GR) -Thioredoxin reductase (TrxR) – dependent on selenium	Mainly dietary antioxidants from plant based healthy foods: -Vitamins: vitamin A, C, E -Trace elements: zinc, selenium (as parts of enzymes) -Carotenoids: β-carotene, lycopene, lutein, zeaxanthin -Phenolic acids: chlorogenic acids, gallic acid, caffeic acid, and others -Flavonols: quercetin, kaempferol, myricetin -Flavanols: proanthocyanidins, catechins -Anthocyanidins: cyanidin, pelargonidin -Isoflavones: genistein, daidzein, glycitein
Nonenzymatic antioxidants: -Glutathione (GSH) -Melatonin -Uric acid -Coenzyme Q -Lipoic acid -Transferrin -Albumin -Lactoferrin -Bilirubin -Ceruloplasmin -Nicotinamide adenine dinucleotide phosphate (NADPH)	

Adapted from Bouayed and Bohn (2010) and Barceló and Barbé (2005).

dependent or independent of mitochondria (Chandra et al., 2000; Sinha et al., 2013). They also play an important role in the initiation and progression of tumors and are additionally related to the induction of cellular senescence (Dreher and Junod, 1996; Lu and Finkel, 2008). Several exogenous factors can be responsible for the generation of ROS, like ultraviolet radiation, cigarette smoking, alcohol, ischemia-reperfusion injury, chronic infections, and inflammatory disorders (Bhattacharyya et al., 2014). Cigarette tar in particular can produce large amounts of H_2O_2 (Nakayama et al., 1989). For this reason, smoking can promote lipid peroxidation of cellular membrane lipids, thus stimulating atherosclerosis and endothelial dysfunction and increasing the risk for lung cancer and cardiovascular diseases (Frei et al., 1991; Santanam et al. 1997; Ambrose and Barua, 2004).

3. Antioxidative effects of melatonin

The potent antioxidant activity of melatonin derives from various complex mechanisms, including direct scavenging of radicals, suppression of prooxidant enzymes, stimulation of antioxidant enzymes, and maintenance of mitochondrial homeostasis. Generally speaking, melatonin provides protective cellular effects, especially for mitochondria, and it prevents the damage of proteins, lipids, and DNA (Zhang and Zhang, 2014). In vivo experiments pointed out that melatonin increases gene expression of multiple antioxidative genes in neuronal tissues, including those of CuZnSOD, MnSOD, GPx, catalase, and glutathione reductase (Kotler et al., 1998; Esparza et al., 2005; Gomez et al., 2005).

There is also evidence that melatonin protects skin from UV injury by enhancing the expression of SOD, catalase, and GPx (Fischer et al., 2013). Some experimental models of tissue damage demonstrate that melatonin acts protectively by reducing oxidative stress and lipid peroxidation (Pieri et al., 1995; Reiter et al., 1998). Furthermore, melatonin exerts anticancer activities through either cytostatic mechanisms or cytotoxic actions by inhibiting cell proliferation and/or reducing viability in several cancer cell lines, including rat pancreatic tumor cells (Gonzalez et al., 2011), human hepatocellular carcinoma cells (Ordoñez et al., 2014), human B-lymphoma cells (Trubiani et al., 2005), human myeloid HL-60 leukemia cells (Rubio et al., 2007), and human neuroblastoma cancer cells (Garcia-Santos et al., 2006).

In addition, melatonin helps to maintain the integrity of the mitochondrial membrane and interacts with the mitochondrial electron transport chain complexes I and IV. For this reason, it promotes electron flux under basal conditions and increases ATP production (Martin et al., 2000, 2002). Melatonin is also able to limit the decline of intramitochondrial glutathione, and related to this it improves ATP production, as well as the electron transport chain activity, by directly detoxifying ROS/RNS. Due to these capabilities, melatonin may protect against ischemia-reperfusion-induced cardiac damage and mitochondrial dysfunction in sepsis (Lopez et al., 2006; Petrosillo et al., 2006).

However, the mechanisms implicated in the regulation of antioxidant enzymes by melatonin in vivo are not precisely established. It is generally agreed that in cultured cells the stimulation of antioxidant enzyme gene expression by melatonin occurs at low to high nanomolar levels (Tan et al., 2014).

Moreover, it was demonstrated that the metabolites of melatonin also possess antioxidant abilities. Melatonin is metabolized through chemical or enzymatic reactions, leading to formation of AMK (N1-acetyl-5-methoxykynuramine), AFMK (N(1)-acetyl-N(2)-formyl-5-methoxykynuramine), and 3-OHM (3-hydroxymelatonin), which have important biological significance (Reiter et al., 2007; Tan et al., 2007; Galano et al., 2013). In particular, 3-OHM was observed to be a more potent antioxidant against $OH^{\bullet}$ and hydroperoxyl ($HO_2^{\bullet}$) radicals as compared to melatonin or its other metabolites (Galano et al., 2014; Tan et al., 2014). In addition, it has been suggested that 3-OHM protects mitochondria against oxidative damage, because it effectively prevents oxidative degradation of cytochrome c by H_2O_2 (Tan et al., 2014). It was also reported that AFMK reduces lipid peroxidation and oxidative DNA damage and that AMK has a major capacity to scavenge ROS (Tan et al., 2001; Ressmeyer et al., 2003). Furthermore, AMK reduces intracellular NO levels by inhibiting NOS activity in the cytosol and in mitochondria (Leon et al., 2006), and both AMK and AFMK exhibit antiinflammatory and immunoregulatory activities (Radogna et al., 2010; Mauriz et al., 2013).

4. Prooxidant actions of melatonin

So far, melatonin has been well known for its ability to act as an antioxidant, and the vast majority of studies have concentrated on analyzing its role as a scavenger of ROS. Nevertheless, recent studies were also able to demonstrate that melatonin can act as a prooxidant under certain conditions (Zhang and Zhang, 2014) (summarized in Table 2).

In 1999 Medina-Navarro et al. tested the antioxidant activity of melatonin in vitro on lipids and erythrocyte membranes against singlet oxygen, as compared with ascorbate and beta-carotene. They showed that at a concentration of 0.5 mM melatonin increased lipid peroxidation of cell membranes, and after 120 min the hydroperoxide concentration was about 5 times greater (35.4 mM) than the maximum concentration reached by the sample in the absence of antioxidants (7.2 mM). In

Table 2. Summary of studies showing prooxidant effects of melatonin.

Effect	Melatonin concentration	Incubation time with melatonin	Cell type	Reference
Lipid, protein oxidation	0.2–0.6 mM	120 min	Erythrocyte membranes	Medina-Navarro et al., 1999
Cell viability↓ GSH↓, ROS↑	0.1–10 μM 1–10 mM	96 h 15 min	HepG2	Osseni et al., 2000
ROS↑, Fas-induced apoptosis↑	0.01–1 mM	30–360 min	Jurkat	Wolfler et al., 2001
Redox active iron↑, heme-oxygenase-I↑	1 mM	5 h	Mouse brain slice	Clapp-Lilly et al., 2001
Cytotoxicity↑, ROS↑	1 mM	48 h	CMK, Jurkat, MOLT-4	Büyükavcı et al., 2006
ROS↑, GSH↓	1 mM	2 and 6 h	U937	Albertini et al., 2006
ROS↑, 5-LOX↑, PLA2↑, arachidonic acid↑,	1 mM	1 min to ~5–6 h	U937	Radogna et al., 2009a; Radogna et al., 2009b
NF-κB↑	1 mM	Up to 5 h	U937	Cristofanon et al., 2009
ROS↑, viability↓, caspase activity↑	1 mM	1–24 h	HL-60	Bejarano et al., 2011
ROS↑	2–25 μM	~20 min	Isolated renal mitochondria	Zhang et al., 2011
ROS↑	2.5–50 μM	~20 min	Human kidney mesangial cells and mitochondria	Zhang et al., 2011
ROS↑, intracellular calcium↑, apoptosis↑	20–100 μM	*	Human platelets	Girish et al., 2013
Ca^{2+} induced mPTP opening↑, nitrites↑, mitochondria ETC↓, parasite toxicity↑	25–50 nM	72 h	Leishmania infantum	Elmahallawy et al., 2014

*: Not evident from the publication.
GSH = Glutathione, ROS = reactive oxygen species, CMK = human megakaryoblastic cell line, MOLT-4 = human lymphoblastoid leukemia cell line, 5-LOX = 5-lipoxygenase, PLA2 = phospholipase A2, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, mPTP = mitochondrial permeability transition pore, ETC = electron transport chain.

addition, application of melatonin in the range of 0.2 to 0.6 mM induced protein oxidation (Medina-Navarro et al., 1999). This was probably the first evidence that melatonin may act as a prooxidant under certain circumstances and further investigations in in vitro cellular systems succeeded.

One of these studies in human leukemic Jurkat cells showed a concentration- and time-dependent increase of intracellular ROS generation and Fas-induced apoptosis by melatonin at concentrations between 10 and 1000 μM, whereas no significant ROS generation at melatonin concentrations of lower than 10 μM was detected (Wolfler et al., 2001).

Osseni et al. observed in vitro that the cell viability in the human liver cell line HepG2 significantly decreased at melatonin concentrations of 0.1–10 μM after 96 h of incubation time. In contrast, at a shorter incubation time of 24 h, antioxidative effects of melatonin were detected with increased intracellular GSH levels and improvement of cell viability. High melatonin concentrations from 1 to 10 mM, however, lead to GSH depletion (Osseni et al., 2000). Another study by Clapp-Lilly et al. in an organotypic slice culture model of Alzheimer disease showed that 1 mM melatonin increased markers of oxidative stress, such as hem-oxygenase-1, as well as redox active iron, while lower concentrations of melatonin below 100 μM resulted in a reduction of oxidative stress (Clapp-Lilly et al., 2001). In addition, Büyükavcı et al. described that higher concentrations of melatonin showed moderate cytotoxic effects in CMK, Jurkat, and MOLT-

4 cells, which were, however, associated with significant ROS production (Büyükavcı et al., 2006). Regarding the human promyelocytic leukemia cell line HL-60, melatonin induced a significant increase of ROS at a concentration of 1 mM after 1–24 h of incubation. The same concentration increased the activity of caspase-9 and caspase-3, which may be related to its prooxidant effects (Bejarano et al., 2011). Cristofanon et al. described that melatonin can induce NF-κB activation in U937 cells and suggested a possible involvement for ROS induced by melatonin (Cristofanon et al., 2009). In contrast to cancer cells, melatonin did not induce cytotoxicity in noncancer cells, including human umbilical vein endothelial cells (Cui et al., 2012), primary hepatocytes (Kojima et al., 1997), neuronal stem cells (Fu et al., 2011), and HT22, a mouse hippocampal cell line (Rodriguez et al., 2013). A further study reported that in human platelets, melatonin considerably increased the generation of intracellular ROS and Ca^{2+}, stimulating mitochondrial membrane depolarization, cytochrome c release, caspase activation, protein phosphorylation, and phosphatidylserine externalization (Girish et al., 2013).

Regarding the incubation time, several studies noted a significant increase of ROS from several minutes to 48 h after the addition of melatonin. It has been, for example, discovered that ROS production in U937 cells increased after 2 and 6 h (Albertini et al., 2006), whereas CMK cells, Jurkat cells, and MOLT-4 cells showed an increase in ROS production after 48 h. Therefore it is suggested that the modulation of ROS production is also cell type-dependent. (Büyükavcı et al., 2006) (Table 2).

Furthermore, one study in HepG2 cells demonstrated that relatively lower concentrations of melatonin (0.1–10 μM) showed antioxidant effects within 24 h and became prooxidant after 96 h (Osseni et al., 2000). Generally, it is important to mention that the prooxidant effects of melatonin have been only reported in in vitro cell culture systems, primarily in cancer cells, and therefore could not be generalized to other cells and systems. Moreover, as was described in the studies above, the evidence suggests that the prooxidant action of melatonin is dependent on concentration, cell type, and incubation time.

5. Potential mechanisms involved in the prooxidant effects of melatonin

One important mechanism through which melatonin may promote ROS generation might involve calmodulin-mediated PLA2 activation, leading to 5-LOX-mediated ROS production (Radogna et al., 2009b). A recent study showed that the calmodulin-specific inhibitor chlorpromazine inhibits ROS production by melatonin in U937 cells. In addition, chlorpromazine prevented the interaction of melatonin with calmodulin, suggesting that the weak interaction between calmodulin and melatonin may be involved in the generation of ROS by pharmacological concentrations of melatonin (Radogna et al., 2009b). Furthermore, Anton-Tay et al. observed that, at physiological concentrations (1 nM), melatonin modulates the subcellular localization of calmodulin in MDCK (Madin-Darby canine kidney) cells (Anton-Tay et al., 1998). Moreover, Sarti et al. noted that within a few hours melatonin causes a transient increase of the expression of neuronal nNOS, which leads to an elevated RNS production in mitochondria. The rise of nNOS causes a mild reduction in oxidative phosphorylation efficiency, paralleled by a depression of the mitochondrial membrane potential and a shift of glycolysis. Interestingly, the induction of nNOS by melatonin was observed only at a physiological level of 1 nM and not at 10 or 100 nM of melatonin (Sarti et al., 2013).

Generation of ROS is probably not dependent on the plasma membrane MT_1/MT_2 receptors, because, for example, the receptor antagonist luzindole does not block melatonin-mediated ROS generation. Furthermore, ROS are not stimulated by a set of MT_1/MT_2 melatonin analogues (Radogna et al., 2009a). The mitochondrial complex III may be a potential site for melatonin-induced ROS generation in cultured primary human mesangial cells and in mice kidney mitochondria (Zhang H et al., 2011; Zhang HM et al., 2011). However, it is not clear whether melatonin directly interacts with the mitochondrial complex III to increase ROS generation.

6. Conclusions

Related to experimental studies that were primarily conducted in different cell lines, it can be assumed that melatonin can also act as a prooxidant under certain circumstances. The relevance of this finding is unclear; both beneficial or harmful effects may apply. Potential beneficial effects may include antiparasitic or anticancer actions. On the other hand, long-term supplementation of melatonin may possibly also dose-dependently exert side effects, like in this case generation of free radicals. Experimental studies showed that antioxidants may also act as prooxidants under certain conditions (Bergstrom et al., 2012), and epidemiological studies in the last years suggest that long-term supplementation with antioxidants like vitamin E may have detrimental effects on health (Bjelakovic et al., 2013). Little is known about the side effects of melatonin. Therefore, clinical studies addressing this issue are needed to estimate potential risks.

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