Melatonin: circadian regulation of biomarkers of oxidative stress

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Summary

Melatonin, released primarily from the pineal gland, controls the 24 hour biological clock. It is now known that melatonin also controls circannual reproductive cycles in animals and plays a role in neuroprotection, tumor-suppression, and immunomodulation. The hormone is also a potent antioxidant. This review is an attempt to document the pleiotropic biological effects of melatonin.

Introduction

Melatonin, the hormone secreted from pineal gland, has been reviewed over the past few decades. After its discovery by Aaron Lerner and his colleagues in 1958 (Lerner et al., 1958), many researchers have worked upon this neurohormone and its physiological and therapeutic significance. Melatonin is ubiquitously found in all living organisms and controls the 24-hour cycle of sleep/wake rhythm. Melatonin has been established as an efficient antioxidant and a major activator of biological clock. A host of other important functions such as control of circannual reproductive cycles in animals, neuroprotection, tumor-suppression, and immunomodulation have been ascribed to this pineal secretion, and these various functions have been reviewed previously (Chakravarty and Rizvi, 2008). While pineal melatonin is as an efficient free-radical scavenger, pharmacological melatonin has also received acceptance as a novel drug against oxidative stress during the past few years (Reiter et al., 2009).

Melatonin and circadian system

Melatonin is synthesized in the pineal gland in response to photic stimulus. The light signals are perceived by the retina and transmitted *via* retino-hypothalamic tract to the suprachiasmatic nucleus (SCN) which is the principal circadian rhythm generator in mammalian body-system. In the absence of light, the clock genes are activated to release melatonin. Once it is synthesized, melatonin is released into the bloodstream directly. Melatonin is also synthesized in minor quantities in other organs which are not the recipients of photic stimuli. Molecular clocks, located in various components of gastrointestinal tract such as liver, stomach, and pancreas are coordinated by the master clock located in the SCN. The complex interplay between the central nervous system (CNS) and peripheral organs in driving circadian rhythm indicates its relation with the metabolic machinery. The peripheral metabolic tissues respond in various ways to metabolic signals, and the CNS coordinates these peripheral events through direct control and indirectly by regulating the food intake and energy expenditure (Green et al, 2008).

Circadian rhythm represents one of the most evident manifestations of adaptation to the environment and its examples include endocrine oscillations in various hormones such as melatonin, cortisol, or growth hormone, as well as rhythms in physiological parameters such as body temperature, and rhythmicity in behaviour as observed in the activity-rest cycles, feeding and the sleep-wake cycles. Circadian pacemakers or clocks are biological oscillators that measure local time and regulate temporal organization of organisms. Properties that distinguish these pacemakers and are required for generating circadian rhythms include: (i) endogenous oscillations, (ii) adjustment by environmental cues such as the light/dark cycle and social interactions, and (iii) the production of an efferent signal that reaches the rest of the organism to set the time in all the oscillators present in the body in an overall synchrony. The retina has fundamental importance in the circadian system because it is responsible for photoreception of the light/dark environmental conditions that synchronize the master clock through its projections to the SCN and other brain structures.

Most photopigments present in the retina are involved in nocturnal and diurnal vision (Aschoff, 1960). A novel group of photoactive molecules, which include opsins and cryptochromes, may participate in circadian photoreception. Several reports support the possibility that, in mammals, melanopsin may be the photopigment involved in mediating the photic entrainment of circadian rhythms. It is in this context that retinal ganglion cells (RGCs) may constitute the retinal cells that participate in circadian phototransduction, regardless of typical rods and cones. This ability of the retina to receive light and detect changes

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in illumination backgrounds affects the circadian system, which is further supported by an endogenous clock mechanism which may be still preserved when isolated from the rest of the organism (Guido et al., 2002).

Once synthesized from the amino-acid precursor, L-Tryptophan, melatonin is released into the blood stream from where it flows along with the circulation to various cellular and sub-cellular compartments. The lipophilic nature of the indoleamine contributes to melatonin's movement across the blood-brain barrier and its efficiency as a free radical scavenger (Hardeland and Pandi-Perumal, 2005). Oxidative stress resulting from free radicals is the outcome of the imbalance between oxidants and antioxidants. It is widely accepted that tissues under oxidative stress are characterised by altered fluidity of cell membranes (Vigh et al., 2007). For proper functioning of the transporter enzymes the maintenance of ionic homeostasis and the optimal fluidity of the cell membrane are essential. Endogenous melatonin has been found to stimulate several antioxidant enzymes and membrane–linked ion channels, which are entrusted with the regulation of ionic imbalance in cells. These proteins, as well as the oxidative free radicals that escalate the pathophysiology of diseases, act as potent biomarkers of oxidative imbalance.

Reactive oxygen species and cellular damage

An increase in the oxidant level in erythrocytes has been reported from experimental evidences related to the antioxidant enzymes (Mates et al., 1999). The availability of molecular oxygen from the atmosphere is possible through the enzymes present in the erythrocytes which readily catalyse the gaseous exchange in the cells. ROS are continuously produced under aerobic conditions; they are involved in several processes, i.e., transformation, regulation or degradation. Nevertheless, ROS concentration is strictly controlled by endogenous antioxidants, thereby protecting living organisms against the potentially deleterious effects of ROS. ROS include superoxide anion radical $(O_2^{\bullet -})^-$ especially produced in cytosol, mitochondria and endoplasmic reticulum-, hydrogen peroxide (H_2O_2) produced in peroxysomes, the highly reactive hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). Toxic oxygen species have been shown to cause DNA-damage, enzyme-inactivation, oxidation of hormones, peroxidation of lipids, and membrane-perturbations followed by cellular degradation. Indeed, a preponderance of evidence has been accumulating to suggest that free-radical reactions are major contributors to cellular breakdown processes (Rice-Evans and Burdon, 1993).

The red blood cells experience oxidative damage at various levels of cellular composition, including membrane-associated changes (Yelinova et al., 1996). Oxidative damage has been shown to change a number of RBC properties. Increased membrane rigidity and decreased RBC deformability can be induced by oxidative cross-linking of spectrin. Oxidative damage can also alter membrane permeability and lead to hemolysis (Lubin and Chiu, 1982). Therefore, RBCs may act as a good model for studies related to oxidative stress. The role of melatonin in the upregulation of such oxidant-affected cellular components has been studied.

The role of melatonin in improving mitochondrial respiratory chain activity and increasing ATP production in different experimental conditions has been reported (Acuna-Castroviejo et al., 2005, 2007). The rhythmically secreted melatonin from the pineal gland of vertebrates is involved in the regulation of circadian and seasonal rhythms (Reiter, 1991). Since melatonin is also produced in different organs and tissues of mammals such as the lacrimal gland, harderian gland, bone marrow, and blood platelets, in minor quantities (Hardeland, 2008; Karasek and Winczyk, 2006), the extrapineal source of melatonin seems to be related to the antioxidant and free radical scavenging properties of the indoleamine, which are expressed in all subcellular compartments including the mitochondria (Reiter et al., 2000, 2002).

Mitochondria are the sources as well as the vulnerable targets of ROS. An oxidative damage to the oxidative phosphorylation machinery will eventually lead to cell death (Shigenaga et al., 1994). Melatonin decreased oxygen consumption concomitantly with its concentration, inhibited any increase in oxygen flux in the presence of an excess of ADP, reduced the membrane potential, and consequently inhibited the production of superoxide anion and hydrogen peroxide (Tan et al., 1993). At the same time it maintained the efficiency of oxidative phosphorylation and ATP synthesis while increasing the activity of the respiratory complexes (mainly complexes I, III, and IV) (Acuna–Castroviejo et al., 2005, 2007). Since melatonin reduces oxygen flux, the increased activity of the respiratory chain suggests an improvement of the respiratory efficiency. Melatonin participates in the physiological regulation of mitochondrial homeostasis and maintains ATP production (Lopez et al., 2009).

Certain enzymes, including catalase, superoxide dismutase , glutathione peroxidase, and glutathione reductase play an important role in antioxidant defense by converting ROS to non-radical products (Vural et al., 2001). Glutathione reductase generates reduced glutathione from oxidized glutathione. Glutathione, in turn, reduces hydrogen peroxide, lipid peroxides, disulfides, ascorbate, and free radicals. Melatonin also stimulates glutathione peroxidase activity, which metabolizes the precursor of the hydroxyl radical, hydrogen peroxide, to water (Okatani et al., 2000**).** Upon oxidation, melatonin converts to a number of antioxidant compounds such as cyclic 3-hydroxymelatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine and N^1-acetyl-5-methoxykynuramine. Therefore, melatonin assumes the role of a broad spectrum antioxidant that is more powerful than glutathione and mannitol in detoxifying free radicals and that can protect cell membranes from oxidative damage more effectively than vitamin E **(**Pieri et al., 1994; Reiter et al., 2009).

Melatonin: an efficient antioxidant

Oxidative free radicals resulting from cellular metabolism tend to disturb cellular membrane integrity and enzyme activities in red blood cells. The plasma membrane is composed of proteins and long chains of polyunsaturated fatty acids which make it vulnerable to oxidative stress. Free radical-induced lipid peroxidation intensifies in the absence of a protective antioxidant defence mechanism resulting in decreased cell membrane fluidity. Malondialdehyde and hydroxynonenal, by-products of lipid peroxidation, are considered as toxic second messengers that can diffuse within or even escape from the cell and attack targets far from the site of production. Under physiological conditions, the reactive oxygen species are eliminated by enzymatic and non-enzymatic antioxidant defence systems. Melatonin counteracts the toxic effects of lipid peroxidation in cell membranes (Catala, 2007). Melatonin owes its free radical-scavenging property to the methoxy group at position-5 of the indole nucleus and the acetyl group of the side chain of melatonin (El-Sokkary et al., 1999). However, melatonin may act as a weak peroxide-radical scavenger, which, therefore, increases the demand for some other cellular mechanism for lipoperoxyl amelioration in red blood cells. Melatonin affects receptors in the genome and has a stimulatory effect on antioxidant enzyme systems such as superoxide dismutase (SOD) and reduced glutathione (GSH) for the purpose (Tomas-Zapico and Coto-Montes, 2005). Ample evidence is in favour of the antioxidative potential and membrane stabilizing properties of melatonin (Garcia et al., 1997; Reiter et al., 2009).

Glutathione, a thiol-containing protein, is an efficient antioxidant present in almost all living cells. The recycling of glutathione in the cells depends on a NADPH-dependent glutathione enzyme-system which lies in the form of an antioxidative circuit. The plasma GSH/GSSG redox state is controlled by multiple processes, which include synthesis of GSH from its constitutive amino acids, cyclic oxidation and reduction involving GSH peroxidase and GSSG reductase, transport of GSH into the plasma and degradation of GSH and GSSG by γ-glutamyltranspeptidase. Upregulation of γ-glutamylcysteine synthetase, a rate-limiting enzyme in GSH synthesis, after melatonin administration has been speculated earlier (Blanco et al., 2007). Melatonin has been found to upregulate cellular glutathione level to check lipid peroxidation in brain cells (Pandi-Perumal et al., 2006**).** Circadian changes in glutathione level in response to exogenous melatonin have been studied. An increase in reduced glutathione content may participate in reduction of lipid peroxidation in erythrocytes (Chakravarty and Rizvi, 2011a**).**

The altered redox balance with pathophysiology of diseases and increasing age may affect the activity of virtually any protein because essentially all proteins contain cysteine and methionine, amino acids that are subject to oxidation - reduction changes. Maintenance of redox state of sulphydryl residues and reduction of lipid hydroperoxides at the expense of electron donors, such as ascorbate and NADH, is essential for normal energy metabolism in the cell. The neutralisation of oxidants also involves some membrane proteins that comprise the plasma membrane redox system (PMRS). Increase in PMRS is a requisite for ionic homeostasis. Melatonin reduces the harmful oxidative species by stimulating other antioxidants. The nocturnal decrease in PMRS is due to the reduced oxidative stress in presence of pineal secretion as well as exogenous melatonin, in a dose-dependent manner (Chakravarty and Rizvi, 2012).

The uninterrupted exchange of cationic species leads to an alteration in cellular pH. The movement of ions is essential for regulating pH and restoring optimal activities in the cytoplasm. Sodium/hydrogen exchanger (NHE) is a membrane –associated protein that exists as several isoforms; NHE3 shows elevated activity in the night for regulating cellular pH during metabolic acidosis. The NHE isoform present in erythrocytes, NHE1, indulges in higher exchanger activity in presence of endogenous melatonin. The nocturnal upregulation is synchronous with the onset of pineal secretion at night. Another membrane-linked protein, sodium-potassium ATPase (sodium pump), maintains electrochemical sodium and potassium gradient

of tissues and cells. It also regulates intracellular calcium homeostasis (Tian et al., 2001). Exacerbated cellular response to oxidative damage involves activation of Na^+/K^+-ATPase. Both the ion-transporters were found to show significant increase in activity by *in vitro* melatonin-treatment in the presence of endogenous (pineal) melatonin (Chakravarty and Rizvi, 2011b). The response to *in vitro* treatment was pronounced at a concentration close to maximal plasma melatonin level. Similar upregulation was reported earlier in the renal antiport proteins (Wang and Huang, 2004)

Acetylcholinesterase (AChE) is a key component of cholinergic transmission. AChE activity in erythrocytes may be considered as a marker of central cholinergic status (Kaizer et al., 2008). Acetylcholinesterase activity has been used as an indicator of activity pattern of human erythrocytes (Prall et al., 1998). Free radicals and increased oxidative stress have been found to reduce AChE activity (Molochkina et al., 2005). The circadian stimulation of AChE activity has been reported (Moudgil and Kanungo, 1973). A higher concentration of melatonin also inhibits AChE activity in red blood cells probably due to the pro-oxidative nature of melatonin. AChE activity depends on the membrane fluidity and surface charge (Klajnert et al., 2004). Membrane fluidity decreases due to oxidative stress, which also decreases AChE activity (Kaizer et al., 2008). Exogenous melatonin modulates the activity of acetylcholinesterase in erythrocytes which is more pronounced at such concentration close to the peak physiological level of melatonin in the body (Rizvi and Chakravarty, 2011).

Higher concentration of melatonin may act as pro-oxidant, thus dampening the normal flow of protons across the cell membrane. It is quite interesting that the total antioxidant status in serum has also been found to display a parallel rise along with the circadian melatonin secretion with highest values exhibited at night (Benot et al., 1998). The duration and dosage of therapeutically administered melatonin may desensitize MT1 and MT2 melatonin receptors thus altering the phase-dependent activation of physiological processes (Dubocovich et al., 2005; Gerdin et al., 2004a, b). The *in vitro* modulation of PMRS by melatonin in erythrocytes can be seen as a receptor-independent mechanism by which the red blood cells can mitigate oxidative stress.

Mechanism of action and other biological effects

The mode of action of melatonin is partially receptor-mediated (Pandi-Perumal et al., 2008). The predominant signalling pathway for melatonin receptors is through inhibition of cAMP formation *via* a pertussis-sensitive G-protein-coupled receptor (Reiter et al., 1997). Several major actions of melatonin are mediated by MT_1 and MT_2 membrane receptors (Wronka et al., 2008; Sallinen et al., 2005). MT_1 and MT_2 function antagonistically to mediate several immunological actions in peripheral organs and cells. A third binding site, MT_3, subsequently characterized as quinone reductase 2, inhibits oxidative stress through elimination of prooxidant quinones (Hardeland et al., 2003; Mailliet et al., 2004; Reiter et al., 2004). The receptors of melatonin are present in brain and other tissues (Morgan and Williams, 1989). These receptors are responsible for chronobiological effects at the SCN, the circadian pacemaker. Melatonin directly binds to calmodulin thereby inhibiting CaM-kinase II, and causing the activation and Ca^{2+}-dependent membrane translocation of protein kinase C and retinoic acid nuclear receptors (RORα1, RORα2 and RZRβ) (Benitz-King et al., 1996; Schrader et al., 1996; Anton-Tray et al., 1998; Pandi-Perumal et al., 2006). MT_1 and MT_2 receptors are expressed in various parts of the CNS and in peripheral organs (Conway et al., 1997). Melatonin synchronizes water transport in kidney-derived epithelial cells through cytoskeletal modification. Melatonin increases stress fibres and focal adhesions in MDCK cells: participation of Rho-associated kinase and protein kinase C against free radicals and hepatic and renal lead – toxicity (El-Sokkary et al., 2005; Benitz-King 2006; Ramarez-Rodriguez et al., 2007). An oral dose of melatonin may cure the reticuloendothelial blockade and renal anaemia in haemodialyzed patients and oxidative stress induced by dialysis. However, the mechanism remains unclear (Labonia et al., 2005, Karasek et al., 2005).

Melatonin may exert a direct influence on the immune system by modulating melatonin receptors interleukin-2/interleukin-2 receptor system (Guerro and Reiter, 2002; Karasek and Winczyk, 2006). Melatonin as well as its implants enhances the production of IL-4 in bone marrow T-helper cells and of granulocyte-macrophage colony-stimulating factor in stromal cells (Pandi-Perumal et al., 2006). It can also protect bone marrow cells from apoptosis (Borjigin et al., 1999). Endogenous and exogenous melatonin modulates lymphocyte proliferation via specific receptors (Wronka et al., 2008). The presence of melatonin receptors and melatonin biosynthesis enzymes in the thymus provides evidence for immunoregulatory role of this hormone (Naranjo et al., 2007). Exogenous melatonin has been shown to counteract immunodeficiencies secondary to viral

diseases, stress events or drug treatment and pathogenesis of autoimmune diseases, particularly in patients with rheumatoid arthritis (Maestroni and Conti, 1993; Pandi-Perumal et al., 2006). In humans, daily oral melatonin administration increases natural-killer cell activity (Del Gobbo et al., 1989). Additionally, melatonin reportedly regulates gene expression of several immunomodulatory cytokines and increases the levels of IL-1β, IFN-γ, and tumor necrosis factor-α and stem cell factor by splenocytes .The nocturnal rise in blood melatonin levels in humans is associated with rise in the thymic production of peptides including thymosin 1a and thymulin (Ravindra et al., 2006).

Discussion and conclusion

Melatonin can penetrate all the morphophysiological barriers in the human body due to its lipophilic and hydrophilic characteristics and has antioxidant effects over a wide area. The administration of melatonin to humans or animals, has not shown evidence of toxicological implications. Melatonin is secreted in the night irrespective of the species concerned. It controls the circadian rhythm and a circannual rhythm in photoperiodic species. The duration of these secretions may affect the reproductive system. An inappropriate time schedule of melatonin administration may elevate the physiological concentrations of the neurohormone and desensitize melatonin receptors. A long duration of exposure to melatonin could also mimic an "artificial darkness" condition, when the body tends to slow the physiological functions for sleep (Guardiola-Lemaitre, 1997). To ensure proper physiological functions circadian rhythm with a basal zero level during the day needs to be conserved.

Furthermore, administration of large doses of melatonin could induce high concentrations of melatonin and of different metabolites that could have deleterious effect on cardiovascular system, central nervous system, sleep-wake cycle, glucose metabolism and even cancer. Pharmacological profiling of melatonin is essential so as to reduce the pro-oxidative activity of the indoleamine that may interrupt its therapeutic efficiency. The knowledge of the fundamental mechanism of action of melatonin, including molecular biology, also needs to be taken into account for evaluation of possible side effects. However, it can be clearly shown that melatonin can control oxidative disturbances at concentrations close to the physiological levels of melatonin present in our body.

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