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Review

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Many roles of melatonin: diversity and complexity of reaction pathways

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Abstract: Melatonin (N-acetyl-5-methoxy tryptamine) is well known as a free radical scavenger and antioxidant involved in different biological and physiological regulation such as modulation of circadian rhythms, seasonal reproduction, retinal physiology and sleep regulation. Synthetic melatonin is available commercially, and its supplements have been used clinically to treat a variety of medical conditions such as jet lag, shift work and sleep disorders. Recent studies demonstrated that melatonin serves as an inhibitor of myeloperoxidase (MPO) under physiological-like conditions. Melatonin-dependent inhibition of MPO occurred with a wide range of concentrations that span various physiological and supplemental ranges. Myeloperoxidase is enzyme involved in leukocyte-mediated host defenses but plays a pathogenic role during chronic inflammatory conditions. MPO levels implicate inflammation in the walls of coronary arteries, which in turn, may indicate a risk for heart disease or heart attack. Thus, supplementary concentrations of melatonin can influence physiological and pathophysiological role of MPO. In addition, MPO modulates nitric oxide production, so melatonin can indirectly affects nitric oxide concentration. Amounting evidence shows new emerging role of melatonin and its metabolites beyond the classic one. This review focuses on newly discovered mechanistic pathways of melatonin activity that has to be taken into consideration when discussing pharmacological uses of melatonin.

INTRODUCTION

The neurological hormone melatonin (*N*-acetyl-5-methoxy tryptamine) was first isolated from pineal gland and identified in 1958 (Lerner, Case and Takahashi, 1958). Hormone role in circadian rhythm was detected soon after that (Amstrong *et al.*1986). In 1991, Ianas *et al.* reported free radical scavenging activity of melatonin but also observed prooxidant activity. Tan *et al.* in 1993 confirmed free radical scavenging activity of melatonin. Since then, a numerous *in vitro* and *in vivo* studies demonstrated ability of melatonin to scavenge reactive oxygen and nitrogen species including hydroxyl radical, superoxide ion, peroxy radicals, singlet oxygen, nitric oxide, peroxynitrate and its metabolites (Reiter *et al.*, 2008;Tan *et al,1993;* Galano *et al.*,2011). Later, important discovery of melatonin ability to stimulate some important antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase followed (Hardeland, 2005) adding another beneficial role of melatonin in regards to oxidative stress. In addition, some research suggested that melatonin can inhibit hydrogen peroxide (H_2O_2)-induced lipid peroxidation and lipoprotein modification. However, the possible *in vivo* reaction pathways for these properties have yet to be identified (Hardeland, 2005 Schaffazick *et al.*, 2005). Classical role of melatonin has been confirmed many times showing melatonin involvement in different biological and physiological functions such as modulation of circadian rhythms (Dawson and Armstrong, 1996), seasonal reproduction (Dardente, 2012), retinal

physiology (Wiechmann and Sherry, 2013) and sleep regulation (Claustrat, 2005). Number of reviews explained, in detail, these functions (Pandi-Perumal *et al.*, 2006) including free radical scavenging activity but antioxidant ability of melatonin drew the most attention. Mitigating harmful effect of oxidative stress by melatonin looked promising for the treatment of a number of diseases although increased flux of free radicals is secondary effect rather than cause of a many pathophysiologies. Synthetic melatonin is available commercially, and its supplements have been used clinically in a variety of medical conditions such as jet lag, shift work, and circadian rhythm sleep disorders, cancer, longevity and antioxidant therapy, sepsis, and neurodegenerative disorders (Kostoglou-Athanassiou, 2013; Srinivasan *et al*., 2013).

However, melatonin is the substance that has not been regulated by the U.S. Food and Drug Administration, and as a dietary supplement is widely available in forms of different preparations with unknown concentrations of active substance. No data about safety and active dose is available. Due to the relatively low physiological levels of melatonin in human tissue, melatonin has been recommended as a supplement for a wide variety of conditions in doses ranging from 0.3 to 1000 mgs/day given for 1-4 weeks. In clinical studies, a wide range of melatonin doses have been used, including "low-dose" (0.1 to 1.0 mgs) for jet lag and insomnia in the elderly, “moderate-doses” (5 and 10 mgs), often taken by mouth 30 to 60 minutes prior to sleep time for disturbances in children with neuro-psychiatric disorders and bipolar disorder, or “high dose” (50 to 1200 mgs) for treating cancer and migraine headaches (Altun and Ugur-Altun, 2007).

One of the issues related to the melatonin beneficial effect is what can be considered as biological and pharmacological concentration. When analyzing this, several issues have to be taken into account:

a. Melatonin is produced in pineal and non pineal sites. Non pineal concentrations can reach micromolar levels while pineal melatonin is generally in pico to nanomolar range.
   However, melatonin is also synthesized non pineal sites such bone marrow (Conti *et al.*, 2000), gastrointestinal tract (Bubenik, 2002), leukocytes (Hardeland *et al.*,2011), lymphocytes (Calvo *et al.*,2013), skin (Slominski *et al.*, 2005), airway epithelium (Kvetnoy, 1999). Melatonin concentration in these cells is much higher than those normally found in the blood and it does not seem to be regulated by the photoperiod. Mounting evidence shows that extrapineal melatonin as a key paracrine signal molecule with significant role in intercellular interactions.
b. Pineal melatonin easily crosses into circulation what is not a case with nonpineal melatonin where only small concentrations are released in circulatory system.
c. Melatonin can be metabolized enzymatically and nonenzymatically in the presence of free radicals. Thus, total oxidative stress has effect on final catabolism of melatonin.
d. Melatonin catabolic products AMFK and AMK are also strong free radical scavengers but of different reactivity towards different free radicals.
e. Melatonin has an immunomodulatory effect and upregulates antioxidant and proinflammatory enzymes (Deng et al., 2006; Rodriguez et al.,2004)
f. Drug interactions also influence the bioavailability of melatonin. (Hartter et al., 2000)
g. Melatonin modulates inflammatory enzyme myeloperoxidase and through the interactions of MPO with nitric oxide synthase regulates production of nitric oxide. (Galijasevic et al., 2008; Galijasevic et al., 2003)
h. General conditions such as age, total health status, antioxidant levels, over the counter and prescribed medications affect outcome of the melatonin therapy

Considering the variety of reactions pathways that melatonin can be involved and multiplicity of intracellular effects in it is clear that its concentration significantly direct outcome of the study especially in a complex biological milieu. In addition, no detailed dose studies have ever been done in relationship with particular condition. Generally, classical view of melatonin as a cronobiotic and antioxidant molecule with submolar circulating concentrations still persists. However this approach is insufficient and several new reactions pathways deserve attention and additional research. A variety of melatonin actions is presented in Figure 1.

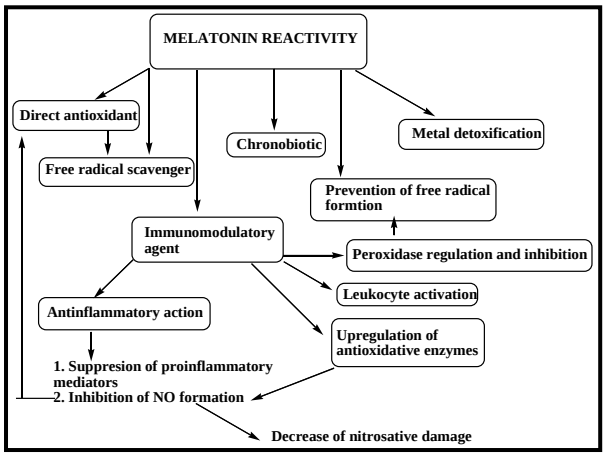

Figure 1. Multiple modes of melatonin actions

Reaction mechanism of melatonin

a. Catabolic pathways

Melatonin synthesized by the pineal gland is released into the bloodstream and then into other bodily fluids, including cerebral spinal fluid (CSF) (Rousseau *et al.*, 1999) saliva, (Vakkuri, 1985) and bile (Tan *et al.*,1999).

Both, endogenous and administered melatonin are generally considered to be metabolized in humans in the presence of cytochrome P450, forming 6-hydroxymelatonin (6-HMEL) followed by sulfate or glucuronide conjugation to 6-hydroxymelatonin sulfate or 6-hydroxymelatonin glucuronide and excreted in urine (Young*et al.,* 1985). However, melatonin is present in many tissues that do not have hepatic cytochromes P450, thus, tissue levels may not necessarily be a function of rates of hepatic metabolism or even directly related to plasma concentrations (Reiter *et al.,* 2003).

Second conversion pathway involves oxidation reaction e.g., by indoleamine 2,3-dioxygenase, myeloperoxidase, oxoferryl-Hb, hemin and a number of free radical such as $O_2^{\bullet-}$, $^{\bullet}OH + O_2^{\bullet-}$ $CO_3^{\bullet-}$,$^1O_2$, O_3, aromatic cation radicals and $O_2^{\bullet-}$, giving one of the most important melatonin metabolites, AFMK (*N*^{1}-acetyl-*N*^{2}-formyl-5-methoxykynuramine). In reaction with hydroxyl radical, melatonin can convert to c3-OHM (cyclic 3-hydroxymelatonin) that in the presence of another hydroxyl radical molecule forms AFMK. Deformylation of AFMK by hemoperoxidase and arylamine formamidase gives AMK (*N*^{1}-acetyl-5-methoxykyunaramine). Interestingly without administration of exogenous melatonin, serum levels of AFMK were undetectably low (Harthe *et al.,* 2003; Rozov *et al.,*2003). Several metabolites of melatonin have been detected in a different tissues, cells and urine (Tan *et al.,* 2003). AMFK and AMK are generally considered as major most important metabolites of melatonin, probably due to their strong free radical scavenging activities. Recent review pointed out that direct free radical scavenging activity may be relevant when concentrations of melatonin are high, and some of the oxidative reactions might be valid when done in isolate system, but questions about potency of this type of melatonin activity in biological systems remain to be investigated more (Hardeland *et al.,* 2008). The multiplicity of enzymatic and nonenzymatic catalysts capable of forming AFMK show the complexity of reactions mechanisms and difficulty to determine exact beneficial melatonin dose. Number of clinical studies showed excellent results in the treatment of different physiological conditions and disease. However, there are also a number of conflicting studies showing no or a little improvement after melatonin therapy. Study investigating efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction concluded that there is no evidence that melatonin is effective in treating secondary sleep disorders or sleep disorders accompanying sleep restriction, such as jet lag or shift work disorder. There is evidence that melatonin is safe with short term use, but additional studies are needed to determine its long-term safety (Buscemi *et al.,* 2006). Del Fabbro *et al.* tested results of prior studies that suggested that melatonin can attenuate weight loss, anorexia, and fatigue in patients with cancer. The primary purpose of their study was to compare melatonin with placebo for appetite improvement in patients with cancer cachexia. In cachectic patients with advanced cancer, oral melatonin, 20 mg, at night did not improve appetite, weight, or quality of life compared with placebo. Singer et al (Singer *et al.,* 2003) conducted a research to determine the safety and efficacy of 2 dose formulations of melatonin for the treatment of insomnia in patients with Alzheimer's disease. No statistically significant differences in objective sleep measures were seen between baseline and treatment periods for the any of the 3 groups: placebo, taking 2.5-mg slow-release melatonin, or 10-mg melatonin. Nonsignificant trends for increased nocturnal total sleep time and decreased wake after sleep onset were observed in the melatonin groups relative to placebo. Year before, Cardinalli *et al.* report the effect of melatonin (4-month-long treatment with 6 mg/day) in 45 AD patients with sleep disturbances. Melatonin improved sleep and suppressed sundowning, an effect seen regardless of the concomitant medication employed to treat cognitive or behavioral signs of AD. Melatonin treatment seems to constitute a selection therapy to ameliorate sundowning and to slow evolution of cognitive impairment in AD patients. Considerable evidence confirmed that melatonin can inhibit LDL oxidation that in turns plays an important role in the development of atherosclerosis [Reiter *et al.,* 2008]. One of the more detailed studies investigated *in vitro* protective effects of melatonin against oxidation of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC). Liposomes and low-density lipoproteins conjugated dienes (CD) and hydroperoxides from cholesteryl esters (CEOOH) and phospholipids (PCOOH) were measured as indices of lipid peroxidation (Marchetti *et al.,* 2011).Melatonin was efficient in lowering lipid peroxidation in LDL, as shown by the decrease in the formation of CDs and hydroperoxides. Authors clearly mentioned that melatonin protections was based on free radical scavenging effect of melatonin since hydroxyl radical was directly involved in LDL oxidation. Another study performed in more complex experimental system showed that daily melatonin supplementation in mice increases atherosclerosis in proximal aorta. This study suggests that caution should be taken as regards high melatonin dosage in hypercholesterolemic patients (Tailleux *et al.,* 2002). Clearly, number of factors mentioned earlier in the text can influence the result of the studies. Multiplicity of reaction pathways and concentration of used melatonin and, in addition, age, physiology of pineal gland, oxidative stress, antioxidative and proxidative enzyme status when clinical studies are in question makes it difficult to obtain definitive answers on beneficial effect of melatonin. The possibility of additional undetected or concentration -dependent reaction mechanisms, either of melatonin or one if its metabolites, adds to the complexity of any type of studies.

b. Metal Chelator

Many studies have investigated link between metal-induced toxicity and carcinogenicity, and connected it to ability of metals to catalyze generation of reactive oxygen and nitrogen species in biological systems. Metal-mediated formation of free radicals results in modifications to DNA bases as well as can enhance lipid peroxidation. Biological studies showed that melatonin can protect against DNA oxidation and lower lipid peroxidation. (Reiter *et al*., 2008). Antioxidative activity of melatonin is generally accepted as one of the

mechanism that alleviates metal induced toxicity and oxidative changes on the system. Melatonin molecule being composed of a 5-methoxyindole group and an N-ethylacetamide group with nitrogen and oxygen atoms has also a potential ability to act as a metal chelator. The metal chelation ability of melatonin is mentioned marginally in literature reviews. In 1998, Limson *et al.* showed that melatonin formed complexes with aluminium (III), cadmium (II), copper (II), iron (III), lead and zinc suggesting metal detoxification role of melatonin in biological system. The metal chelating ability of melatonin was concentration dependent (Limson *et al.*, 1998; Gulcin *et al.*, 2002). Clearly, complex formation is dependent on amounts of available metal ions that can vary in different tissues and on melatonin concentrations. As a result, different melatonin concentrations are necessary for the complete formation of stable complexes that have significant consequences if melatonin is used as a detoxification agent in biological systems. Interestingly, experiments with iron showed that melatonin can bind iron (III) not to iron (II) thus blocking generation of free radical via the Fenton reaction. Parmar *et al.* found that melatonin affords some protection to rat hepatocytes in the presence of copper. Electrochemical studies show that melatonin, in addition to binding Cu^{2+}, may provide protection against copper-mediated free radical damage by binding Cu^{1+}. Lack *et al.* showed that melatonin form complexes with lithium, potassium, sodium and calcium. The stability of the complexes formed between melatonin and the metals decreased with the metals as follows: $K^{+}>Li^{+}>Na^{+}>Al^{3+}>Ca^{2+}$ .Another study evaluated the metal chelating and hydrogen peroxide (H_2O_2) scavenging activity of melatonin (Gulcin *et al.*, 2003). The metal chelating activity increased with increasing concentrations of melatonin (20–60 μg/mL). Based on these results, it is concluded that melatonin is an effective metal chelating agent. Interestingly, most of the studies showed the ability of melatonin to bind metal ions but a definitive mechanism is not known yet. One possible mechanism, in a complex biological milieu, is simple scavenging activity of free radicals like hydroxyl radicals generated in vivo from the metal-catalyzed breakdown of hydrogen peroxide, according to the Fenton reaction (Liochev and Fridovich 2002). However, O'Halloran and co-workers recently reported that the upper limit of so-called "free pools" of copper was far less than a single atom per cell (Rae *et al.*, 1999). This data imply that Fenton like reaction when copper is present is not feasible under these conditions. Thus, possibility of direct interaction of melatonin with metal ions forming complexes is another valid mechanistic pathway. We recently showed by using UV and IR spectroscopy direct formation of Cu-Melatonin complex on solutions where hydrogen peroxides was not present thus excluding free radical formation (Galijasevic, 2013). This study is initial one, done with varying copper and melatonin concentrations in order to find conditions necessary for the complex formation. Further experiments in biological systems need to be done to test whether chelation reactions is possible in more complex system. Another interesting metal that has deleterious effect on cells is mercury. Study showed that a 24-hour exposure to 50 μg/L mercury induced significant cell cytotoxicity in neuroblastoma cells (Galijasevic *et al.*,2000). Treatment of cells with melatonin before administration of mercury greatly reduced the mercury-induced cytotoxicity. Mechanism is not known but authors suggested that either chelation activity is present or melatonin causing production of increased levels of intracellular antioxidants such as glutathione .It is not excluded that both these mechanisms could be operating simultaneously.

More detailed studies are needed in order to confirm metal chelating abilities of melatonin, including experimental conformation of formed complex, determination of ligand donor atoms and molecular geometry of formed complex. In addition, kinetic of complex formation and determination of complex electrochemical potentials would give definitive answer on mechanism of melatonin interactions with metals. However, those results would not imply that exact mechanism exist in biological systems, simply because of complexity of studied systems and melatonin antioxidant activity and as a new research shows different enzymes inhibitory activity.

c. Enzyme inhibitor

Myeloperoxidase (MPO) is one of the most abundant enzymes in neutrophils and monocytes, involved in leukocyte-mediated host defenses. It is also thought to play a pathogenic role under certain circumstances such as during inflammatory tissue injury and chronic inflammatory conditions (Nicholls and, Hazen, 2004). MPO has been recently reported to be useful for identifying inflammation in the walls of coronary arteries, which in turn may indicate a risk for heart disease or heart attack (Weiss, 1988). Thus, inhibiting MPO may be a key step in the prevention of pathophysiology of LDL oxidation. MPO uses H_2O_2 generated during a respiratory burst as co-substrate to form cytotoxic oxidants and diffusible radical species. Evidence suggests that MPO-mediated reactive oxidants can promote protein nitration, lipid peroxidation, amine chlorination, and thiol nitrosylation. Recent review summarized catalytic cycle of MPO and corresponding inflammatory injury by a different mechanism (Arnhold and Flemming, 2010). At plasma levels of halides, chloride (Cl^{-}) is a major co-substrate for MPO and the cytotoxic oxidant, hypochlorous acid (HOCl), is produced. In addition to HOCl, MPO can generate a variety of reactive oxidant species, multiple distinct protein and lipid oxidation products, which have been identified in tissues associated with atherosclerosis and other inflammatory conditions (Shishehbor and Hazen, 2004). The ground state (secreted) form of the enzyme, reacts in a rapid and reversible manner with H_2O_2 to form Compound I. This redox intermediate oxidizes halides via a single two e^{-} oxidation step to their respective hypohalous acids. Alternatively, Compound I may oxidize multiple substrates through two sequential one e^{-} steps forming Compound II and ground state enzyme, respectively. Enhancement in peroxidase catalysis due to reduction of MPO-Compound II has been noted with a series of physiological reductants like superoxide ($O_2^{\bullet-}$), melatonin, tryptophan, nitric oxide (NO), and ascorbic acid (Lee *et al., 1991;* Hallingbäck *et al.*, 2006; Kettle

and Candaeis, 2000). Oxidation of melatonin was first observed by activated neutrophils in a reaction involving myeloperoxidase (Silva *et al.,* 2000). In order to clarify if melatonin is a substrate of MPO, Allegra et al investigated the oxidation of melatonin by MPO redox intermediates, compounds I and II (Allegra *et al.,* 2001). Spectral and kinetic analysis revealed that both intermediates compound I and compound II oxidize melatonin via one-electron processes. Authors concluded that that the rate of oxidation of melatonin is dependent on the H_2O_2 concentration and is not affected by superoxide dismutase. Another study proposes that melatonin serves as potent inhibitor of MPO under physiological-like conditions (Galijasevic *et al.,* 2003). In the presence of Cl^-, melatonin inactivated MPO at two points in the classic peroxidase cycle through binding to MPO to form an inactive complex, melatonin-MPO-Cl, and accelerating MPO compound II formation, an inactive form of MPO. Inactivation of MPO was mirrored by the direct conversion of MPO-Fe(III) to MPO compound II without any sign of compound I accumulation. This behavior indicates that melatonin binding modulates the formation of MPO intermediates and their decay rates. Melatonin-dependent inhibition of MPO occurred with a wide range of concentrations that span various physiological and supplemental ranges. More importantly, the oxidized form of melatonin, N^1-acetyl-N^2-formyl-5-methoxynuramine (AFMK) has no effect on MPO catalytic activity, but functions as a potent antioxidant due to its ability to serve as free radical scavenger. This interplay between MPO and melatonin may have a much broader application in biological systems. Thus, inactivation of MPO and its catalytic cycle can be controlled effectively by melatonin supplementation. Indeed, when the melatonin concentration is less than twice the H_2O_2 concentration, H_2O_2 consumption proceeds in a slower and linear manner and MPO returns to its ground state after melatonin oxidation. This behavior clearly demonstrates that MPO is capable of restoring its catalytic activity and rejoining the peroxidase cycle after melatonin exhaustion. On the other hand, when the melatonin concentration is greater than twice the H_2O_2 concentration, the initial slow phase of H_2O_2 consumption remains at the same rate through the progression of the reaction and ceases when H_2O_2 is completely consumed. Clearly, melatonin concentrations used in inflammatory event controls reaction pathways and inhibition of MPO activity that in turns regulates HOCl production and possible deleterious effect on the biological environment. Effect of melatonin inflammatory events by MPO inhibition has the broader consequences on pathophysiologies where nitric oxide is involved. Nitric oxide is directly involved in MPO catalytic mechanism by a number of different pathways modulating MPO activity and contributing to the detrimental effect of MPO system at the sites of enzyme expression. NO serves as both a ligand and a substrate for MPO, and the overall effect of NO on the catalytic activity depends on the affinity of MPO for NO vs. H_2O_2 and their concentrations. Another mechanism of interactions between MPO and iNOS suggest that the MPO system consumed NO released by iNOS during steady-state catalysis, thereby preventing the NO-induced inhibition attributed to the formation of the iNOS–nitrosyl complex. Thus, removal of NO from the iNOS milieu by the MPO system during steady-state catalysis causes a significant increase in iNOS catalytic activity. Myeloperoxidase, acting as a sink for NO efficiently activates iNOS preventing shutdown of the NO production system (Galijasevic et al., 2003). Numbers of studies have shown that melatonin influence the bioavailability of NO by inhibition of NO synthase activity (Aydogan *et al.,* 2006). Besides enzyme inhibition melatonin, AMFK, and AMK can scavenge NO and peroxynitrite. The highest reactivity towards radical nitrogen species has AMK, but its formation dependents on a number of factors mentioned earlier. Melatonin dose of 1 microM-1mM inhibits NO production in immunostimulated macrophages mainly by inhibiting the expression of iNOS. (Gilad *et al.,* 1998). Tested concentrations are very high even for a nonnpinela sites of melatonin production, and inhibition can only be achieved when pharmacological doses of melatonin are taken. Even in that case, it is not clear whether such concentrations can be achieved at the needed sites due to the variety of melatonin reaction pathways. Another study confirmed too that, besides its antixodant activity, melatonin inhibits peroxynitrite formation by inhibition of the enzyme nitric oxide synthase in some brain tissues (Leon *et al.,* 2004). Experiments in vivo showed that melatonin administration prevents sepsis-induced electron transport chain damage decreasing the activity and expression of INOS and mtNOS, thus reducing intramitochondrial nitric oxide (NO) and peroxynitrite (ONOO-) levels (Acuña-Castroviejo *et al.,* 2003). Melatonin improved vascular function in experimental hypertension, reducing intimal infiltration and restoring NO production. Melatonin improved the NO pathway also in animal models for the study of diabetes and prevented NO down-regulation and adhesive molecules up-regulation in nicotine-induced vasculopathy (Rodella *et al.,* 2013). The question about most preferred reaction mechanism of melatonin still remains unanswered. Whether melatonin concentrations have an effect on a preferred mechanism is still unknown too. In addition, presence of myeloperoxidase at the sites of inflammation and interplay between MPO, NO and iNOS adds to the complexity of melatonin reactions pathways.

At the sites of inflammation melatonin can act as free radical scavenger of already formed NO and $ONOO^-$, inhibit NOS production, act as a substrate for MPO compounds I and II or inhibit MPO and block H_2O_2 consumption and modulate interplay between MPO and iNOS, thus indirectly affecting production of NO. Which mechanism will be predominating one, or what can act as an efficient switch between possible reaction pathways is not experimentally shown yet. Interactions of iNOS, MPO and free radicals at the sites of inflammation with melatonin are presented in Figure 2. Presented reaction pathways are not intended to be comprehensive and only major reactions are shown due to the clarity.

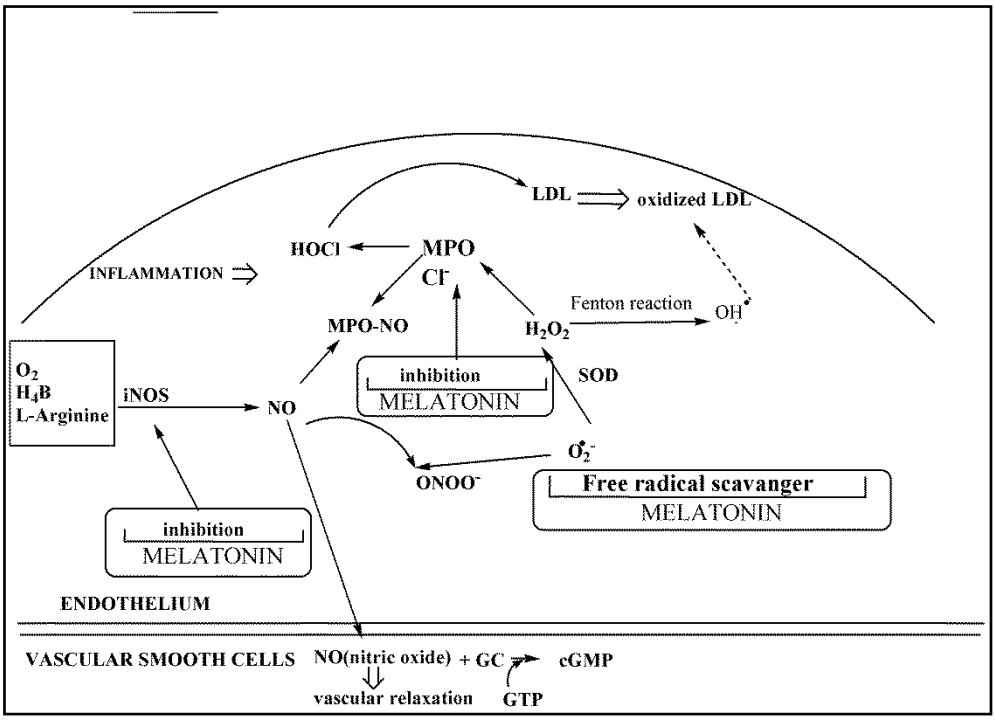

Figure 2. Involvement of melatonin multiple reaction pathways at the sites of inflammation. Melatonin can exert its effect by free radical scavenging, myeloperoxidase inhibition, nitric oxide syntahase inhibition or acting as a substrate for myeloperoxidase. Each action will result with a cascade of reactions with a different outcome

CONCLUSION

Melatonin like no other molecule drew attention as a natural supplement with a numerous beneficial effects. Most of these claims are based on a melatonin ability to scavenge harmful free radicals. Research shows that melatonin reactivity comprises many different reaction pathways. It is reasonable to conclude that different conditions of system that is treated melatonin and other reactants concentrations, and melatonin metabolites availability influence the final outcome. Some of the most reactions pathways such as modulation of inflammatory enzymes and possible metal detoxifications deserve more attention. Definitive dose-response studies are needed if beneficial effect of melatonin is going to be established. In addition, concentration and type of free radicals that can actually be scavenged by melatonin need to be tested and compared to the antioxidant present in the biological system Melatonin is remarkable molecule with a variety of biological roles showing a promising role in a treatment of variety conditions and diseases justifying additional systematic research.

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Summary/Sažetak

Melatonin (N-acetil-5-metoksi-triptamin) je poznat kao skupljač slobodnih radikala i antioksidans koji sudjeluju u različitim biološkim i fiziološkim regulacijama kao što su modulacija cikardijarnog ritma, sezonske reprodukcije, fiziologija retine i regulacija sna. Sintetski melatonin je komercijalno dostupan, a njegovi su dodaci klinički korišteni za liječenje različitih medicinskih stanja kao što su jet lag, smetnje u radu i poremećaji spavanja. Nedavne studije pokazale su da melatonin služi kao inhibitor mijeloperoksidaze (MPO) u fiziološkim uvjetima. Melatonin inhibira MPO pri različitim koncentracijama što može utjecati na fiziološku i patofiziološku ulogu MPO. Osim toga, MPO modulira proizvodnju nitrogen oksida, tako da melatonin može neizravno utjecati na koncentraciju nitrogen oksida. Brojni dokazi pokazuju novu ulogu melatonina i njegovih metabolita pored klasične uloge . Ovaj rad analizira novootkrivene mehanističke puteve aktivnosti melatonina koje treba uzeti u obzir pri primjeni melatonina u farmakološke svrhe.