Melatonin reduces oxidative/nitrosative stress due to drugs, toxins, metals, and herbicides

Russel J. Reiter, Ahmet Korkmaz, Sergio D. Paredes, Lucien C. Manchester, Dun-Xian Tan

Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas, USA

Correspondence to: Prof. Russel J. Reiter, Ph.D., Dr.h.c.mult.
Department of Cellular and Structural Biology
University of Texas Health Science Center
7703 Floyd Curl Drive, San Antonio, Texas 78229 USA
e-mail: reiter@uthscsa.edu

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Abstract

The purpose of this brief review is to introduce the reader to the vast amount of published literature related to the free radical scavenging and antioxidative activity of melatonin. However, this review summarizes only a few of the many conditions in which melatonin has been found to be protective against oxidative/nitrosative damage. Melatonin ameliorates the extensive free radical-mediated damage that ensues following exposure to a wide variety of environmental insults. Concisely reviewed in this report are the protective effects of melatonin against toxic prescription drugs, neural toxins, herbicides and metals. The findings have clear implications for the utility of melatonin in toxicology.

INTRODUCTION

Many drugs, toxins, pesticides, herbicides and metals cause molecular and cellular damage because they generate a variety of reactive agents known as free radicals and related reactants [4,7,19]. These damaging agents include both oxygen-based (reactive oxygen species or ROS) and nitrogen-based reactants (reactive nitrogen species or RNS). The resulting tissue destruction is referred to as oxidative or nitrosative stress, respectively. The most damaging ROS is the hydroxyl radical (•OH) while the most destructive RNS is the peroxynitrite anion ($ONOO^-$) [25]. These two brigands account for a significant portion of the molecular damage that ensues in response to exposure to the toxic substances mentioned above.

Protection against oxidative/nitrosative damage is provided by antioxidants. These can be either direct free radical scavengers, metal chelators, or they are enzymes which metabolize radical products to innocuous molecules. There are a large number of endogenous molecules that function as direct free radical scavengers. Enzymes in this category that destroy radicals include the superoxide dismutases, glutathione peroxidase, etc.

In the following brief survey we review the mechanisms by which melatonin functions to reduce oxidative/nitrosative stress. Moreover, we summarize data documenting the efficacy of melatonin in neutralizing the effects of a number of toxic agents.

MELATONIN AS AN ANTIOXIDANT

Melatonin (N-acetyl-5-methoxytryptamine) is a remarkably efficient protector against damage inflicted by ROS/RNS. This ability stems from the fact that melatonin, i), directly scavenges a variety of radicals and radical products, ii), stimulates several antioxidative enzymes, iii), promotes the synthesis of another important antioxidant, glutathione, by stimulating its rate limiting enzyme, gamma-glutamylcysteine synthase, iv), reportedly binds metals thereby preventing their participation in free radical formation, and v), stimulates the activity of mitochondrial complexes and prevents free radical generation [10].

Beyond these actions, melatonin has other features that make it important as an antioxidant. Thus, in addition to being endogenously produced it is also ingested in the diet (all plants investigated to date reportedly contain melatonin). Furthermore, melatonin has access to every cell in the organism and its movement is not constrained (as with some antioxidants) by morphophysiological barriers, e.g., the blood-brain barrier. Also, within cells melatonin neutralizes radical products in mitochondria, the cytosol and also in the nuclei of cells [11].

Even though melatonin was only discovered to be an antioxidant in 1993 [26], a very large number of publications have documented its ability to reduce oxidative/nitrosative stress. Finally, not only is melatonin itself an effective neutralizer of free radicals, but likewise many of its derivatives have a similar functional capacity.

HERBICIDES

The bipyridyl herbicides, paraquat and diaquat, are notorious for their toxicity to plants where they are involved in redox cycling. Likewise, in bacteria and animals this mechanism accounts for their damaging actions. Among a variety of possibilities, one target of paraquat is DNA [21].

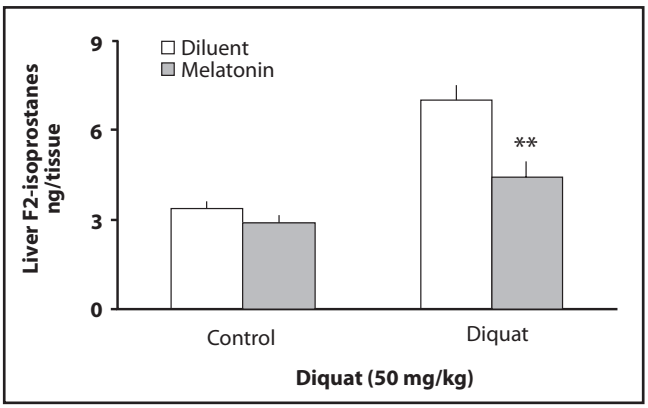

Figure 1. Levels of hepatic F2-isoprostanes, measured by gas chromatography/negative-ion chemical ionization/mass spectrometry, in mice 6 hours after a control injection (0.9% NaCl) or diquat with or without melatonin co-treatment. F2-isoprostanes are prostaglandin-like compounds that result from free radical-catalyzed peroxidation of arachidonic acid. **p<0.001.

The herbicide paraquat is especially toxic to the lungs since several respiratory cells take up this agent against a concentration gradient. Also, the lung has a high concentration of oxygen lending itself to elevated oxidative stress. For diquat, lung is not a major target but rather the intestine and liver suffer the greatest damage.

Shortly after melatonin was discovered to be an antioxidant, Melchiorri and co-workers [17,18] tested its ability to reduce the peroxidation of lipids in the lungs of rats treated with a high dose of paraquat. After paraquat (50 mg/kg) administration, levels of malondialdehyde and 4-hydroxyalkenals (lipid peroxidation products) were elevated in the serum and lungs of rats; these changes were abolished by co-administration of melatonin (5 mg/kg). Similarly, melatonin reversed loss of reduced glutathione concentrations. Finally, melatonin co-treatment with paraquat raised the LD_{50} of the rats from 75 mg/kg to 251 mg/kg paraquat.

Similar observations were made when diquat was used in lieu of paraquat. Thus, Xu *et al.* [29] reported that 50 mg/kg diquat severely damaged the liver and kidneys of mice as evidenced by the rise in hepatic and renal F2-isoprostance levels and serum concentrations of alanine aminotransferase, an enzyme which escapes from damaged hepatic cells into the serum. Melatonin (20 mg/kg), given 30 min in advance of diquat treatment, counteracted each of the changes measured after diquat-only treatment (Figure 1). Moreover, melatonin reduced the acute 24 hour death rate in diquat-treated mice from 91% to 57%.

METALS

The pathologies associated with iron (e.g., hemachromatosis) or copper (e.g., Wilson's disease) overload are well known. As a result of unusually high levels of these metals, free radicals are generated in excess and individuals with these conditions exhibit elevated lipid peroxidation endproducts and protein carbonyls while conventional antioxidants, e.g., vitamins C and E, are typically depressed in these subjects. Thus, oxidative/nitrosative stress is considered a contributory factor to these diseases. Moreover, aluminum has been implicated in the pathogenesis of Alzheimer's disease presumably also because the metal exaggerates free radical generation.

Experimentally, those metals against which melatonin has been found to be protective in terms of reducing oxidized products are summarized in Table 1 [1,3,6,8,13,20]. In most cases the number of publications on each of the metals in relation to melatonin is small and the endpoints few. Uniformily, however, the findings are consistent with a reduction of metal-promoted free radical damage when melatonin is present. The reader is reminded that besides having numerous conventional actions by which it intervenes in reducing damage normally inflicted by ROS/RNS [27], melatonin also reportedly binds several of the metals listed in the table which would reduce their ability to participate in reactions that generate free radicals [14].

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Table 1. A list of metals which are know to be toxic in vivo and against which melatonin has been found to be protective. Melatonin's efficacy in reducing this toxicity probably involves its ability to bind some of these metals as well as to neutralize free radicals that they produce. While each of these metals are believed to generate free radicals in vivo, the mechanisms whereby they do so is widely varied and often not well established. Uranium is a radioactive element that produces free radicals via several means.

aluminum	iron	titanium
arsenic	lead	vanadium
cadmium	mercury	molybdenum
chromium	uranium	nickel
copper	cobalt	

NEURAL TOXINS

There are a very large number of toxins that induce damage to a variety of organs, most often because of their efficacy to exaggerate ROS/RNS generation. A synthetic molecule 1-phenyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to produce Parkinson's disease (PD) in young humans who used heroin contaminated with MPTP. MPTP readily crosses the blood-brain barrier where it is taken up by glial cells and is oxidized to the methyl-phenyl-pyridium ion (MPP^+); it is discharged from the glial cells and is taken up by dopaminergic neurons. MPP^+ is highly neurotoxic and depletes neurons of dopamine causing the rapid onset of Parkinson-like signs. MPP^+ toxicity, especially at the mitochondrial level, stems from its ability to generate nitric oxide and ROS which damage and kill dopaminergic neurons.

MPTP is a highly useful toxin for producing Parkinson-like signs in experimental animals and it is a widely-used model to test for drugs that may be useful as a treatment for PD [16]. Given its mechanism of action, i.e., a mitochondrial poison that promotes electron leakage and free radical generation, many workers have tested melatonin's ability to reduce MPTP/MPP^+ toxicity at the level of substantia nigra (dopaminergic neurons) of the brain. One of the most compelling studies is that of Antolin and colleagues [2]; this group injected low doses of MTPT daily for 35 days into rats. In half of the animals, the daily MPTP injection was preceded by administration of melatonin. As expected, MPTP only caused dramatic reductions in both tyrosine hydroxylase (TH) immunoreactivity and Nissl-stained neurons in the substantia nigra, changes associated with the development of PD. TH is the rate-limiting enzyme in dopamine synthesis. In animals in which melatonin was co-administered with MPTP, TH levels and the number of Nissl-stained neurons were preserved as in control rats. This dramatically documents the ability of melatonin to reduce the free radical toxicity of MPTP at its major target, i.e., dopaminergic neurons. The findings also suggest melatonin would be a potentially useful agent to delay the onset or reduce the severity of PD.

Besides MPTP, 6-hydroxydopamine and rotenone are toxins that have proven useful in inducing Parkinson-like neural damage in experimental models. Both agents are believed to create damage as a result of their free radical generating ability; thus, it is not surprising that the neural toxicity of both 6-hydroxydopamine [24] and rotenone [15,23] is attenuated by melatonin.

PRESCRIPTION DRUGS

Drugs used as medications frequently have negative side effects. This toxicity often limits their use and/or their dose. If the collateral toxicity of these agents could be at least partially ameliorated, it is possible that they could be used at higher doses, and, therefore, probably with greater efficacy. A 2002 review of this subject [22] summarizes the publications related to this issue and based on the findings of these reports, it was clear that combining melatonin with a variety of prescription drugs reduces their toxicity without compromising their effectiveness. In some cases melatonin actually increased the efficacy of the drugs with which they were combined. Since the publication of that review, many additional papers have been published with the results of these studies consistently revealing that melatonin is a highly effective add-on therapy for many prescription drugs. Some examples are summarized in the subsequent paragraphs.

Tissue plasminogen activator (t-PA) is a thromolytic agent commonly used to dissolve blood clots that are obstructing blood vessels, e.g., during a stroke. Obviously, it is essential that obstructed vessels be opened as quickly as possible after an occlusion occurs and intravenous thrombolysis with t-PA is an important and commonly used means of achieving this. In addition to its beneficial effects in ensuring reperfusion, however, t-PA also has some unfavorable actions [28]. To test whether the harmful effects of t-PA could be reversed by combining it with melatonin, Kilic *et al.* [12] obstructed the middle cerebral artery of mice with an intraluminal thread placed at the origin of the middle cerebral artery (MCA) for 90 min. While this intervention obviously led to extensive brain injury, the damage was aggravated by the administration of t-PA. In particular, t-PA activated inducible nitric oxide synthase (iNOS) and decreased phosphorylated Akt levels although it did not change Bcl-X_L expression or caspase-3 activity. Co-treatment with melatonin inhibited t-PA mediated iNOS activity, restored phosphorylated Akt levels and increased Bcl-X_L expression while reducing caspase-3 activity. Thus, the results suggest that melatonin would be a beneficial add-on therapy to reduce the harmful effects of t-PA.

Chen *et al.* [5] also pointed out the shortcoming of using t-PA beyond the three hour therapeutic window in individuals suffering with stroke; they note that t-PA increases the risk of hemorrhagic transformation and potentiates ischemic neuronal injury. In an attempt to resolve this problem, they treated mice with photothrombolytic occlusion of the distal MCA with t-PA alone or in combination with melatonin. In support of the findings of Kilic *et al.* (2005), they reported that melatonin improved post-ischemic preservation of the blood brain barrier permeability and reduced the risk of adverse hemorrhagic transformation that is a common consequence of t-PA therapy for stroke. The conclusion of these authors is that melatonin should be considered as an add-on therapy to reduce the harmful effects of t-PA.

Cisplatin (cis-diamminedichloroplatinum) is a widely used chemotherapeutic agent which is limited by its considerable toxicity. It is well documented that the damaging effects of cisplatin are a consequence of the generation of free radicals in a variety of organs. Considering the antioxidant potential of melatonin, it is natural to assume that this indoleamine would help to alleviate at least some of the side effects of cisplatin. In primary cultures of rat renal tubular epithelial cells, Fukutomi and co-workers [9] observed ROS generation and DNA fragmentation were enhanced when the cells were treated with cisplatin. Of particular interest was the elevated generation of the highly toxic •OH by the drug. When melatonin was added to the medium that also contained cisplatin, •OH generation was markedly attenuated and DNA fragmentation was inhibited. Clearly, melatonin in this study highly significantly reduced damage to the renal tubular epithelium that is normally associated with cisplatin toxicity.

There are numerous additional studies which have confirmed melatonin's substantial efficacy in limiting the toxicity of a host of widely used prescription drugs. Since these studies have also shown that melatonin does not interfere with the beneficial actions of the drugs, its use as a supplemental therapy would seem judicious.

CONCLUDING REMARKS

This brief survey considers only a few of the many reports related to the role of melatonin in protecting against free radical-generating molecules. The literature in this area is massive despite the fact that melatonin has only been known to be an antioxidant for roughly 15 years. There is virtually no toxin against which melatonin has not been found to be beneficial and it is anticipated that future studies will document that melatonin functions as a safeguard against many more damaging agents that exist in the environment. Considering its broad spectrum of antioxidative actions and its remarkable lack of side effects, it should become an important molecule in toxicology.

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