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REVIEW

PHARMACOLOGICAL UTILITY OF MELATONIN IN REDUCING OXIDATIVE CELLULAR AND MOLECULAR DAMAGE

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Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. R.J. REITER, D.-X. TAN, E. GITTO, R.M. SAINZ, J.C. MAYO, J. LEON, L.C. MANCHESTER, VIJAYALAXMI, E. KILIC, Ü. KILIC. Pol. J. Pharmacol., 2004, 56, 159–170

This review briefly summarizes the actions of melatonin in reducing molecular damage caused by free radicals and associated oxygen- and nitrogen-based reactants. All the mechanisms by which melatonin is protective of such a wide variety of molecules, i.e. lipids, proteins, DNA, etc., and in such widely diverse areas of the cell and different organs are likely not yet all identified. Melatonin actions that have been identified include its ability to directly neutralize a number of toxic reactants and stimulate antioxidative enzymes. Furthermore, several metabolites that are formed when melatonin neutralizes damaging reactants are themselves scavengers suggesting that there is a cascade of reactions that greatly increase the efficacy of melatonin in stymying oxidative mutilation. Suggested, but less well defined, processes which may contribute to melatonin's ability to reduce oxidative stress include stimulation of glutathione synthesis (an important antioxidant which is at high concentrations within cells), reducing electron leakage from the mitochondrial electron transport chain (which would reduce free radical generation), limiting cytokine production and inflammatory processes (actions that would also lower toxic reactant generation), and synergistic effects with other classical antioxidants (e.g. vitamins C, E and glutathione). Clearly which of these multiple mechanisms contribute to melatonin's high efficacy in curtailing oxidative damage remains to be clarified. Likewise, it is possible that the key action of melatonin in reducing molecular damage induced by oxygen and nitrogen-based metabolites remains to be identified. Finally, the review summarizes some of the large amount of data documenting the ability of melatonin to limit molecular and organ damage in two situations, i.e. ischemia-reperfusion and ionizing radiation, where free radicals are generally conceded as being responsible for much of the resulting tissue destruction.

Key words: *melatonin, antioxidant, oxidative stress, ionizing radiation, ischemia-reperfusion injury, free radicals*

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Introduction

The discovery of melatonin as a direct free radical scavenger [80] and as an indirect antioxidant *via* its stimulatory actions on antioxidative enzymes [65, 68] has greatly increased interest in the use of this agent in the experimental and clinical setting. Its potential utility in humans is supported by its very low toxicity [59], its availability in a pure form and the fact that it is inexpensive.

Beyond its antioxidant activities, melatonin has been tested for and successfully used in other clinical situations. Perhaps it was initially taken by transmeridian travelers to quell the severity of jet lag [12]. Thereafter, it became popular as a sleep-promoting agent [11] and interest in its use in the suppression of growth of certain cancer types is supported by experimental and clinical observations of a number of scientists [6, 48]. More recently, melatonin has been used as an adjunct treatment in newborn infants suffering with gram-negative bacterial infections [25] and respiratory distress syndrome [26, 27]. Both of these serious conditions are believed to be linked to massive toxic free radical generation and the associated tissue damage [28].

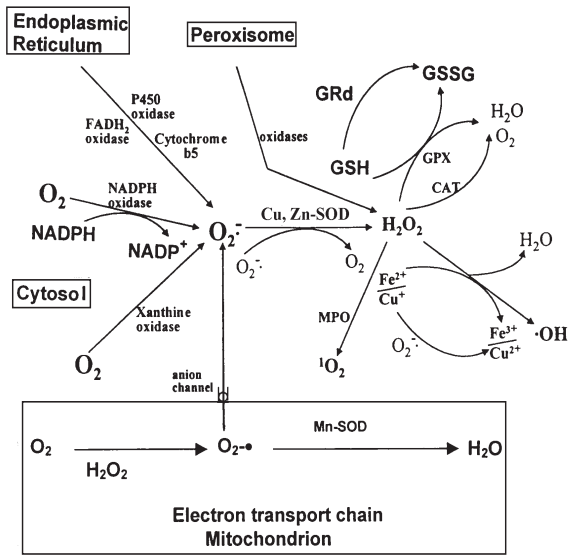

Fig. 1. Molecular oxygen (O_2) is reduced to several free radicals and related reactive species as illustrated in this figure. While the superoxide anion radical ($O_2^{\bullet -}$) is not considered especially reactive, the hydroxyl radical (•OH) readily damages any molecule in the vicinity of where it is produced. A variety of enzymes cause the formation of the $O_2^{\bullet -}$ and, likewise, because of electron leakage from the electron transport chain, radicals are abundantly produced in mitochondria. Several enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) metabolize these reactants to either less toxic or non-toxic by-products. When hydrogen peroxide (H_2O_2) is metabolized by GPX, reduced glutathione (GSH) is oxidized to glutathione disulfide (GSSG) which is converted back to GSH by the enzyme glutathione reductase (GRd)

The following brief review will summarize some of diseases and conditions where free radicals and related reactants are believed, at least in part, to be causative. The molecular damage that is caused by these toxic reactants is often referred to as oxidative stress and the accumulation of these functionally impaired molecules contributes to physiological deterioration and disease development. Additionally, the free radical theory of aging espouses that the accumulation of injured, essential molecules also accounts for tissue deterioration during aging [34]. The current report also summarizes the successful experimental use of melatonin to deter the molecular decay that results from the persistent decline in physiology which is a consequence of the incessant bludgeoning by free radicals and related toxic reactants. Obviously, such an abbreviated summary cannot do justice to the very large number of publications that have appeared in this field; as a result, the reader should consult more extensive reviews on the subjects in which they have a major interest [2, 18, 32, 33, 53, 56, 59, 61, 62, 73, 86, 95].

Oxygen metabolism and oxidative stress and melatonin as an antioxidant

Oxidative damage is a consequence of the inefficient utilization of molecular oxygen (O_2) by cells. The bulk of the O_2 absorbed by cells is used for mitochondrial generation of energy in the form of ATP [1]. A small percentage of the O_2 taken into cells, however, escapes conventional metabolism and is reduced to radicals and non-radical products (Fig. 1) which, because of their high reactivity, are damaging to subcellular structures. Free radicals are molecules or portion of molecules that possess an unpaired electron in their valence orbital. This electron deficient state makes these agents highly reactive and they damage adjacent molecules by abstracting an electron from or donating an electron to them. While the damage to lipids, proteins and DNA seems to be of greatest interest, the injury that occurs is not restricted to these large molecules. Quite the contrary, the reactants generated abuse any molecule in the vicinity of where they are produced. This is particularly true of the most

reactive radicals, e.g. the hydroxyl radical (•OH), which due to its very short half-life interacts with the first molecule it encounters. Because of this, the molecular injuries that occur are described as being site specific.

Radical and radical products formed from O_2 are referred to as being oxygen-based. Additionally, however, there are also highly devastating agents which are nitrogen-based, e.g. nitric oxide (NO•) and the peroxynitrite anion ($ONOO^-$); the latter is formed when the superoxide anion radical ($O_2^{\bullet -}$) couples with NO•. Finally, hypochlorous acid, although not a free radical, can be highly damaging and is identified as a chlorine-based reactant.

Fortunately, organisms are endowed with a series of agents that can either directly detoxify radicals or their associated reactants (collectively referred to as free radical scavengers) or they metabolize them to innocuous molecules (e.g. by antioxidative enzymes). Evidence has accumulated that melatonin is both a direct free radical scavenger [2, 60, 87] and an indirect antioxidant because of its ability to promote the activities of a variety of antioxidative enzymes [65, 68]. While the direct free radical scavenging actions of melatonin are receptor independent, the indirect antioxidative functions may well be mediated by receptors, either located in the membranes of cells or intracellularly.

Besides melatonin, however, there are a large number of other molecules that function as efficient antioxidants, the best known of which include vitamin E, vitamin C and β-carotene. Preliminary evidence suggests that melatonin works synergistically with these important antioxidative agents [29].

As mentioned above, there are a host of conditions where free radicals and associated toxic reactants are either proven or assumed to contribute to the disease state/condition. Some of these are tabulated in Table 1. While this list is not complete, it provides the reader with an idea as to why there is currently extensive interest in the potential use of antioxidants to ameliorate these diseases/conditions. Considering the rather short interval (i.e. a decade) that melatonin has been known to be an antioxidant, it has been remarkably widely tested for its capacity to reduce free radical-mediated damage.

The efficiency of an antioxidant to neutralize a toxic reactant depends on several factors. Besides the ease with which it donates an electron (a common mechanism for detoxification of radical species), its distribution within the cell is equally important. Recall, as noted above, the most reactive reactants (e.g. •OH) travel only a few molecular diameters before they interact with another bystander molecule. Thus, for an antioxidant to prevent this damage it must essentially be at the site where the •OH is generated. Clearly then, the unique solubility of an antioxidant determines its efficacy. As a consequence, lipid soluble antioxidants such as vitamin E are particularly effective in directly detoxifying radicals in the lipid-rich environments of the cell, i.e. cellular membranes. Vitamin E is, however, less effective in protecting nuclear DNA from oxidative mutilation because of the aqueous environment in the nucleus. Conversely, vitamin C, which is readily water soluble, is highly beneficial in the aqueous regions of the cell, particularly the cytosol, and less so in membranes.

In this regard, melatonin seems to be somewhat unusual. While it is clearly a lipid soluble agent, it seems also capable of entering the aqueous environments of the cell. This apparently amphiphilic nature allows melatonin to be protective of membranes, cytosolic molecules and nuclear DNA from

Table 1. Of the large number of situations/disease processes where free radicals and related reactants are believed to be involved in the associated molecular destruction, only some of those in which melatonin has been tested as a protective agent are listed in this table. In specific categories, e.g. drugs, many toxic agents have been examined and virtually without exception melatonin has been found to limit tissue damage from these agents [66]. Also, the authors realize that there is overlap between some of the categories, e.g. chemotherapeutic agents and drugs and ischemia/reperfusion injury and organ transplantation; however, both are listed because of their general importance and to illustrate the broad protective effects of melatonin

Aging	Hyperglycemia
Anoxia	Inflammation
Bile acids	Ionizing radiation (see text)
Carbon monoxide	Ischemia/reperfusion (see Table 2)
Chemical toxins	Mitochondrial toxins
Chemotherapeutic agents	Models of Alzheimer's disease
Cholesterol/homocysteine	Models of Huntington's disease
Drugs	Models of Parkinson's disease
Experimental hyperthyroidism	Neurotoxins
Gastrointestinal ulceration	Organ transplantation
Heavy metals	Phosphine
Hyperbaric hyperoxia	Ultraviolet radiation

free radical damage. The published literature where melatonin has been used *in vivo* documents its ability to reduce molecular injury to each of these macromolecules [64].

As with other antioxidants, what has been difficult to determine is what portion of the protection melatonin provides is a consequence of its direct free radical scavenging ability and what percentage is due to other actions of the indole, e.g. stimulating antioxidative enzymes [65, 68], or reducing electron leakage at the mitochondrial level, thereby reducing free radical generation [1], etc.

Another feature that possibly increases the efficacy of melatonin in reducing oxidative stress is that the metabolites which are produced during the scavenging actions of melatonin, i.e. cyclic 3-hydroxymelatonin (cyclic 3-OHM), N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMF) seem also to be efficient scavengers [19, 67, 81, 87]. Thus, the second and third generation metabolites of melatonin may well contribute to the ability of the parent molecule to protect against oxidative stress. Because of this, rather than scavenging a single radical, melatonin *via* an antioxidant cascade may neutralize a number of toxic reactants [33, 87]. While such measurements are difficult to make, theoretically at least these assumptions are supported by available data.

Comparisons of the relative efficacies of melatonin with classic antioxidants in protecting against oxidative damage have been carried out. Less so under *in vitro* conditions but especially *in vivo*, melatonin has consistently proven to be more effective than vitamins E and C in reducing molecular damage that occurs under high free radical generating conditions [49, 50, 69, 87].

A proposed obstacle to melatonin being a relevant physiological antioxidant is its reported low concentration within organisms or within cells. It is often assumed that melatonin is in equilibrium within organisms and the basis of reference for its concentration is its level in blood. Even at night when maximal secretion of melatonin from the pineal gland occurs, blood concentrations are in the low nanomolar range. This value is very much lower than the levels of other antioxidants such as vitamin C or the intracellular antioxidant, reduced glutathione (GSH). However, it is now apparent that melatonin levels in other bodily fluids or tissues may in fact be orders of magnitude higher than blood concentrations. This became readily apparent when melatonin was measured in fluids such as the bile [84] and cerebrospinal fluid of the third ventricle [77].

Likewise, it has been preliminarily estimated that mitochondria, a major site of free radical generation, also contain much higher concentrations of melatonin than exist in the serum at the same time [52]. Furthermore, besides the pineal gland, a variety of other cells have the capacity to synthesize melatonin, e.g. photoreceptor cells of the retinas and enterochromaffin cells in the gut [9]. In these specific cells and in the adjacent areas, the concentrations of melatonin are again much higher than levels found in the blood. Exactly, how many different cell types have the capability of producing melatonin remains unknown but there may be many [78]. When these cells generate melatonin it may be used within these cells or as an autocrine or paracrine secretion [82]. It is well established that the levels of melatonin in the blood are primarily of pineal gland origin [58] or, under some limited conditions, derived from the gastrointestinal tract [35]. The point of this discussion is that melatonin concentrations in the blood should not be used as an index to judge the concentrations of the indole in other bodily fluids or in subcellular organelles [63]. Theoretically, it is possible that many cells are capable of melatonin synthesis [78] and its production may be induced under high free radical generating conditions, thereby making it readily available intracellularly to combat oxidative damage to essential molecules.

Despite the discussion of the extra-pineal generation of melatonin, it has been shown that even the amounts of melatonin secreted by the pineal gland contribute to free radical protection. Thus, removal of this source of melatonin by surgical pinealectomy has been shown repeatedly to exaggerate the amount of molecular destruction resulting from high free radical states [41, 43, 70–72].

In summary, melatonin's high efficacy in reducing oxidative damage may involve both receptor-independent as well as receptor-mediated processes. In addition to direct free radical scavenging, antioxidative functions of melatonin may include synergistic actions with classic antioxidants [29], stimulation of the synthesis of the important intracellular antioxidant GSH [90], its unique intracellular distribution, the fact that second and third generation metabolites of melatonin are also effective scavengers, its ability to induce gene expression

and activities of antioxidative enzymes [65, 68], its ability to reduce free radical generation at the mitochondrial level [1], as well as yet undefined actions. It is clear that while melatonin is protective against oxidative stress, the mechanisms whereby it achieves this high level of protection requires more extensive investigation.

Tests of melatonin's ability to reduce oxidative stress

Obviously, due to the very large number of reports that have appeared within the last decade which relate to the beneficial actions of melatonin under high oxidative stress conditions, all the publications cannot be cited in the current review. Rather, a few conditions where the clinical utility of melatonin may be more obvious and where the amount of experimental data is abundant were selected for inclusion. The reader is referred to other reviews for more in-depth summaries of the findings [15, 61, 62, 86].

Ischemia/reperfusion injury

Ischemia/reperfusion (I/R) occurs when the blood supply to an organ or region is temporarily interrupted. Most often, I/R injury is discussed relative to the cardiovascular system, i.e. heart attack, or the central nervous system, i.e. stroke or brain attack. While both these conditions can have serious debilitating consequences and often cause death, I/R in any organ is a matter of grave concern.

That the molecular and cellular damage resulting from I/R involves destructive free radicals and related reactants is not contested. While interrupting the blood supply to an organ (depriving it of oxygen and essential nutrients) is very serious and leads to tissue death, it must be quickly relieved; re-establishing blood flow to the deprived tissue, i.e. reperfusion, is, however, also highly damaging. When oxygenated blood re-enters tissues that have been deficient in O_2 for even a brief period, numerous oxygen-based reactants are generated initiating damage beyond that caused by the ischemia. Thus, both ischemia and reperfusion contribute to tissue loss and organ dysfunction. Given that free radicals are widely accepted as being involved in I/R injury, melatonin has been widely tested for its ability to attenuate the tissue damage resulting from transient occlusion of the blood supply to organs.

In terms of the brain, both focal and global I/R methods have been employed to examine the ability of melatonin to reduce tissue damage [15]. The brain is highly susceptible to I/R injury and the consequences are often incapacitating. Although acute thrombolysis and defibrinogenation are effective procedures in selected patients with stroke [42], the possibility of hemorrhagic complications is significant. A large variety of potential neuroprotective agents have been shown to be effective in experimental models of stroke but they have almost universally been shown to be ineffective against stroke-induced neurological damage in humans. Besides the conventional free radical damage that occurs during I/R, excitotoxicity and altered calcium ion homeostasis play major roles in tissue destruction associated with temporary interruption of the blood supply to the CNS. Melatonin, in numerous studies, has been shown to reduce neuronal and glial loss due to *in vivo* excitotoxicity [4, 8, 15, 16, 51].

Additionally, melatonin highly significantly reduced the death of rats in the 5-day interval following kainate (an excitotoxic agent) administration [30, 31]. In this study, the percent of dead animals was reduced from 75% to 7% by melatonin. Judging from the lower levels of oxidized products in the brain of animals suffering with experimental stroke, the authors of the papers cited above universally concluded that melatonin's direct and indirect antioxidative actions likely accounted for its ability to ameliorate kainic acid-induced damage. Besides lowering the death of rats treated with the excitotoxic agent kainic acid, melatonin markedly reduced pyramidal cell loss in the hippocampus of these animals [83]. The pyramidal cells of the hippocampus are especially vulnerable to destruction by kainic acid (and the neurotransmitter glutamate) and are commonly destroyed in the brain of Alzheimer's and Parkinson's disease patients, thereby likely contributing to the memory deficits these patients experience [86].

In experimental models of focal I/R in brain, both physiological levels and exogenously administered pharmacological concentrations of the indole have been shown to afford protection against the resulting molecular damage. Thus, pinealectomy, which lowers endogenous levels of melatonin, enhances neural damage after focal I/R injury (middle cerebral artery occlusion or MCOA) in rats [38]. In this study, where focal I/R injury was achieved by transient occlusion of both common carotid arteries, infarct volume was larger and the number of apoptotic neurons was greater in the

brain of pinealectomized rats compared to these parameters in pineal intact (non-melatonin deficient) animals. These results were quickly confirmed in a subsequent study. Again, the relative melatonin deficiency associated with surgical removal of the pineal gland exaggerated brain damage and neurological disability after endovascular MCAO in rats [43].

In addition to testing the effects of a relative melatonin deficiency on the neurological lesions produced by I/R injury, both Joo et al. [38] and Kilic et al. [43] also administered melatonin to some rats to determine whether giving doses of melatonin that raised blood concentrations of the indole to above endogenous levels, i.e. to pharmacological concentrations, would reduce the amount of neural destruction produced in these experimental stroke models. In these studies, melatonin (2.5 or 4.0 mg/kg) reduced infarct volume by 40% when the indole was given at both ischemia and reperfusion onset. In another study, giving melatonin at a higher dose (20–24 mg/kg) reduced cortical and striatal infarction volumes by 60% and 30%, respectively [8]. Furthermore, melatonin ameliorated the neurobehavioral deficits, enhanced glial cell survival and lowered neural lipid peroxidation caused by temporary ischemia followed by reperfusion of the rat brain. Finally, by examining diffusion-weighted magnetic resonance images of the I/R brain, it was estimated that the amount of cortical edema, and less so, striatal edema, was highly significantly reduced when melatonin treatment was employed [45, 89].

At the molecular level, melatonin has also been shown to protect against neural damage during periods of transient ischemia followed by reperfusion with oxygenated blood. In a recent study [15], melatonin, besides limiting infarct volume, decreased both DNA double and single strand breaks and enhanced cell viability in the penumbral area of the infarct. Also, Bcl-2 induction was enhanced in the ischemic brain after melatonin treatment. Finally, melatonin treatment up-regulated the expression of excision repair cross-complementing factor 6 mRNA, a gene important for nuclear excision repair, in damaged neurons of the penumbra. These findings are consistent with the protective actions of melatonin on neuronal elements during I/R injury.

The number of publications cited above are only a small sample of the large number of reports that have confirmed melatonin's ability to reduced cellular and molecular damage in the brain of animals subjected to experimental stroke. More complete summaries of the data documenting melatonin's high efficacy in limiting anatomical destruction and neurobehavioral deficits in experimental animals after focal or global I/R injury have been published [15, 56, 59–61].

As mentioned above, the consequences of I/R injury in any organ involves essentially the identical pathophysiological mechanisms and includes the generation of massive amounts of free radicals and related reactants with the associated tissue failure and loss which compromizes the function of an organ. Given that melatonin ameliorates the extensive damage that occurs in the I/R brain, it would be expected that the indole would likely similarly lower the loss of cardiac tissue after experimentally induced heart attack. This has, indeed, been shown to be the case.

The bulk of these studies have been performed using the Langendorff heart model. The first study in what is now an extensive series is that of Tan et al. [85]. In the Langendorff heart, a ligature was placed around the descending coronary artery (for 10 min); this reduces coronary blood flow by > 25%. During reperfusion the experimental hearts experienced premature ventricular contractions (PVC) and ventricular fibrillation (VF), features that normally cause death of the organism. When melatonin was given at the time of reperfusion both the PVC and the VF were significantly reduced. The arrhythmias observed in these studies were defined according to the Lamberth Conventions. In this study, Tan et al. [85] also compared the relative efficacies of melatonin and vitamin C in reducing the cardiac pathophysiology caused by I/R. At a dose 500 times greater than melatonin, vitamin C was much less effective in limiting PVC and VF in this I/R model.

Numerous follow-up reports have confirmed the ability of melatonin to ameliorate cardiac damage resulting from a transient interruption of the blood flow to the heart [62, 79]. In one of the most positive reports, Lagneux et al. [46] describe melatonin's ability to protect the heart from I/R as being "spectacular". The bulk of the studies that have examined the protective actions of melatonin at the level of the heart have used pathophysiological endpoints [47, 62]. Also, while in many of the publications pharmacological doses of melatonin were used to quell the electrical disturbances in cardiac

Table 2. In addition to limiting ischemia/reperfusion damage in the brain and heart, as discussed in the text, the following organs have also been shown to be protected from damage during the interruption of their blood supply followed by reoxygenation. In most cases, the actual number of studies is greater than the number of citations listed in the table

Organ subjected to ischemia/reperfusion	Reference documenting the protective effects of melatonin
Fetus	[96]
Gastrointestinal tract	[10, 17, 20]
Kidney	[74]
Liver	[14, 54, 76]
Lung	[36]
Pancreas	[37]
Retina	[13, 41]
Testes	[55]
Urinary bladder	[75]
Vasculature	[5, 45, 89]

muscle after I/R, several have shown that merely lowering endogenous melatonin levels (due to surgical removal of the pineal gland) also exaggerates cardiac arrhythmias that occur after interruption of the blood flow to the heart [70, 71]. Additionally, in one of these *in vivo* studies, the incidence of mortality was increased to 63% of the pinealectomized rats compared to only 25% in the pineal-intact animals.

A recent report by Dobsak et al. [21] further documents the pivotal role of melatonin in limiting myocardial pathophysiology in the I/R rat heart. In this case, an isolated working heart model was used to test melatonin's ability to reduce myocardial damage after transient ischemia followed by reoxygenation. At pharmacological concentrations, melatonin reduced the levels of malondialdehyde (MDA, a product of lipid peroxidation) and apoptotic cell death in the working heart subjected to I/R. As in the earlier reports, the authors concluded that the antioxidant properties of melatonin account for its ability to preserve myocardial function during I/R injury. They also suggest that melatonin be tested clinically as an agent to protect against cardiac damage and dysfunction in human heart attack patients.

Whereas the number of reports confirming the beneficial effects of melatonin in the heart when the cardiac blood supply is compromised are numerous [62], this is not the only situation in which melatonin has been shown to be protective of cardiomyocytes. Also, in the case of drugs, e.g. doxorubicin, that damage the heart muscule, melatonin also reduces molecular damage and limits the toxicity of these otherwise useful agents [22, 64, 66, 72].

Besides the brain and heart, melatonin has been effective in reducing molecular and morphological damage and in preserving normal physiology in other organs during I/R (Tab. 2). Collectively, the experimental data uniformly support the use of melatonin to combat I/R injury.

Ionizing radiation-induced injury

The biological consequences of ionizing radiation are attributable to chemical changes in biological molecules that are a result of energy absorption. Matter absorbs energy from high-energy electrons commonly used in radiation therapy; this results in ionizations. Ionization produces highly reactive species, i.e. free radicals. Subsequent free radical reactions result in damage to the structure of biomolecules thereby altering their function (Fig. 2). The damage that results from direct ionization of the target molecule by radiation are referred to as direct effects while the destruction of molecules by reactions with radiolytic products is referred to as being indirect. The free radicals that are generated during ionizing radiation exposure mutilate a variety of molecules with this accounting for the massive tissue destruction and pathophysiology that can occur.

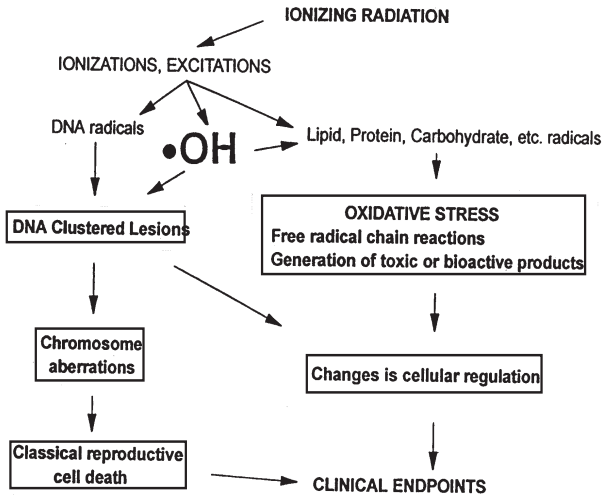

Fig. 2. Ionizing radiation is highly damaging because it generates a number of reactants, especially the highly toxic hydroxylradical (•OH). The resulting damage, as summarized in this figure, can lead to a number of diseases and even cellular and organismal death

Given that ionizing radiation damage involves free radicals, it is not surprising that melatonin has been examined as a potential radioprotective agent.

In vitro studies designed to test the efficacy of melatonin in reducing molecular destruction due to ionizing radiation were carried out by Vijayalaxmi et al. [91–95]. In these studies human blood lymphocytes were treated with one of several concentrations of melatonin for 20 min in advance of being exposed to 1.5 Gy gamma radiation. The findings showed that the addition of melatonin to the incubation media reduced, by roughly 60%, the incidence of genetically-damaged cells as indicated by fewer exchange type of aberrations, acentric fragments and micronuclei in the lymphocytes compared with cells that were treated with diluent only. Importantly, in these studies melatonin did not induce sister chromotid exchanges in the lymphocytes.

The positive results in these studies prompted an *in vivo*/*in vitro* investigation in which human volunteers consumed orally 300 mg melatonin; blood lymphocytes were then recovered from these individuals 1 and 2 h after melatonin ingestion and the cells were exposed to 1.5 Gy gamma radiation [95], stimulated with mitogens and incubated for 48 or 72 h. In the lymphocytes collected from control subjects who did not consume melatonin, there were significant increases in chromosomal aberrations and an increased number of micronuclei; in contrast, lymphocytes collected from individuals who consumed melatonin had much less genetic damage. The greatest protective effect was seen in the blood samples collected 2 h after melatonin ingestion. While it was presumed that melatonin's protective effects related to its ability to scavenge the •OH (which are massively generated during ionizing radiation), it is also likely that melatonin's ability to limit genetic damage in these studies related to its indirect actions in activating antioxidative enzymes.

Other workers have also documented melatonin as a radioprotective agent. Blinkenstaff et al. [7] confirmed the radioprotective actions of melatonin when they showed that administering the indole to mice in advance of a lethal dose of ionizing radiation significantly increased their survival. Specifically, exposing mice to 9.5 Gy ionizing radiation resulted in 100% mortality within 12 days; conversely, melatonin reduced the death rate of the irradiated mice to 43%. A similar survival study in mice documented the ability of melatonin to reduce mortality when the animals were exposed to 8.15 Gy ionizing radiation ($LD_{50/30}$ dose that kills 50% of mice in 30 days) [91].

More recently, several groups have confirmed the radioprotective actions of melatonin. Thus, Badr et al. [3] observed that the frequency of micronuclei in polychromatic erythrocytes and the number of chromosomal aberrations in spermatogonia and spermatocytes were significantly reduced in melatonin-pretreated mice compared to those in similarly-irradiated mice treated with diluent. Likewise, Karbownik et al. [40] as well as Koc et al. [44] reported that melatonin, given in advance of whole body exposure of rats to ionizing radiation, lowered both 8-hydroxy-2-deoxyguanosine (evidence of DNA damage) levels and reduced membrane rigidity (evidence of lipid peroxidation). Most recently, Yasuz et al. [97] and Taysi et al. [88] documented the radioprotective actions of melatonin by showing that the indole greatly limited fractionated irradiation-induced epiphyseal injury in a weaning rat model and in the liver of adult rats, respectively. In the former study, the endpoints were primarily morphological, i.e. limb loss, limb abnormalities and growth arrest. The authors noted that melatonin may be beneficial in growing children who require radiation of an extremity for the purpose of killing malignant tumor cells. The extensive literature regarding melatonin's ability to reduce ionizing radiation damage has recently been reviewed by Vijayalaxmi et al. [95] and Karbownik and Reiter [39].

Concluding remarks

The data summarized herein are only a small sample of the massive literature which documents the ability of melatonin to function as an antioxidant and to reduce oxidative mutilation of essential molecules. The discovery of melatonin as a protective agent against free radicals and related reactants was almost a serendipitous finding. Likewise, it is unlikely that the high efficacy of melatonin in reducing oxidative stress was anticipated but considering the extremely wide variety of circumstances in which melatonin has shown to have antioxidant functions [22–24, 57], its inclusion in the antioxidant arena cannot be denied. On the other hand, it is unlikely that all of the actions by which melatonin reduces free radical damage have been uncovered. Given the widely-protective actions of

melatonin under conditions of elevated oxidative stress, more extensive studies on its beneficial actions in humans are warranted.

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