MINIREVIEW

Antioxidative Effects of Melatonin in Protection Against Cellular Damage Caused by Ionizing Radiation (44547)

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Abstract. **Ionizing radiation is classified as a potent carcinogen, and its injury to living cells is, to a large extent, due to oxidative stress. The molecule most often reported to be damaged by ionizing radiation is DNA. Hydroxyl radicals (•OH), considered the most damaging of all free radicals generated in organisms, are often responsible for DNA damage caused by ionizing radiation. Melatonin, N-acetyl-5-methoxytryptamine, is a well-known antioxidant that protects DNA, lipids, and proteins from free-radical damage. The indoleamine manifests its antioxidative properties by stimulating the activities of antioxidant enzymes and scavenging free radicals directly or indirectly. Among known antioxidants, melatonin is a highly effective scavenger of •OH. Melatonin is distributed ubiquitously in organisms and, as far as is known, in all cellular compartments, and it quickly passes through all biological membranes. The protective effects of melatonin against oxidative stress caused by ionizing radiation have been documented in *in vitro* and *in vivo* studies in different species and in *in vitro* experiments that used human tissues, as well as when melatonin was given to humans and then tissues collected and subjected to ionizing radiation. The radioprotective effects of melatonin against cellular damage caused by oxidative stress and its low toxicity make this molecule a potential supplement in the treatment or cotreatment in situations where the effects of ionizing radiation are to be minimized.**

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Ionizing radiation is classified as a potent carcinogen (1–4). When interacting with living cells, it causes a variety of changes depending on exposed and absorbed dose, duration of exposure and interval after exposure, and susceptibility of tissues to ionizing radiation. The sensitivity of cells to ionizing radiation depends especially on the rate of differentiation and accompanying factors of the tissue as well as on the efficiency of the intrinsic antioxidative defense systems (3, 5–7).

Biological Effects of Ionizing Radiation Due to the Generation of Free Radicals

Radiation injury to living cells is, to large extent, due to oxidative stress (1–9). Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen (O_2) react with cellular macromolecules (i.e., nucleic acids, lipids, proteins, and carbohydrates) (10) and damage them. The interaction of ionizing radiation with living cells induces a variety of reaction products and a complex chain reaction in which many macromolecules and their degradation products participate. The assumption that destructive processes initiated by ionizing radiation begin exclusively in a single subcellular organelle is questionable.

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Major biomarkers of oxidative damage to living cells are (i) lipid peroxidation (LPO) products, comprising volatile hydrocarbons measurable in exhaled air, such as ethane and penthane, and isoprostanes and aldehydic products measurable in tissues and body fluids; (ii) DNA-hydroxylation products (e.g., 8-hydroxy-2′-deoxyguanosine (8-OHdG)) and microscopic indices of damage such as chromosomal aberrations and micronuclei; and (iii) protein hydroxylation products such as oxidized amino acids (11, 12).

Oxidative Damage to DNA. Ionizing radiation is a well-established carcinogen due to the resulting oxidative damage (1–3), and the molecule most often reported to be damaged by this physical agent is DNA. Interactions of ionizing radiation with DNA consist of the direct ionization of DNA (direct effect) (13) and its reaction with surrounding water molecules (the indirect effect), followed by DNA destruction by the induced radicals ($^{\bullet}OH$, $e^{-\bullet}$ and, to much lesser extent, $H^{\bullet}$). A wide variety of biochemical consequences of radiation-induced damage to DNA (5) occurs because of free-radical attack (14).

About 60%–70% of cellular DNA damage produced by ionizing radiation is estimated to be caused by $^{\bullet}OH$, formed from the radiolysis of water (8). $^{\bullet}OH$ are considered the most damaging of all free radicals generated in organisms (15). All biological molecules are targets for $^{\bullet}OH$, and this radical reacts with them at a very high rate, exceeding 10^9 M^{-1} s^{-1}. Thus, the intracellular diffusion distance of $^{\bullet}OH$ is on the order of Ångstroms (16), and the damage it produces is essentially at the site where it was generated. Of the DNA damage produced by $^{\bullet}OH$, oxidized bases, abasic sites, DNA-DNA intrastrand adducts, DNA single and double strand breaks, and DNA-protein cross-links are the most significant (17). These occur primarily by interaction of free radicals with DNA bases and, to a lesser extent, with DNA sugars (14).

Among bases and, generally among nucleic acid components, guanine is the most susceptible DNA target for oxidative reactions mediated by $^{\bullet}OH$ and other free radicals, and it exhibits the lowest ionization potential (17). Thus, one of the most mutagenic lesions, and the most abundant lesion formed in irradiated chromatin is 8-hydroxyguanine (18–21). Once formed, this product can be repaired by several mechanisms (22). Under laboratory conditions, one of the most common and abundant measurable oxidative DNA base adducts is 8-OHdG. This is reported to be a key biomarker related to carcinogenesis (23). The increased oxidation of guanine bases after exposure to ionizing radiation has been observed in numerous studies as well as in different tissues and species (18, 23–25).

The interaction of free radicals with sugar moieties leads to the cleavage of the sugar-phosphate backbone of DNA followed by single-strand breaks that undergo repair processes relatively easily (1). On the other hand, double-strand breaks have more serious consequences. Double-strand breaks are well correlated with the cytotoxic effects of ionizing radiation and are considered the primary lesion involved in cellular death (26).

If DNA repair mechanisms, which are induced after exposure to ionizing radiation, are inefficient, the damaged DNA strands that are copied during replication lead to mutagenesis and carcinogenesis (3). The damaging effects of ionizing radiation lead to cell death and are associated with an increased risk for numerous genetically determined diseases (27).

Among other indices of DNA damage caused by ionizing radiation are chromosome aberrations and micronuclei formation; these are apparent when irradiated cells are observed microscopically (28–33).

Oxidative Damage to Biological Membranes. Besides DNA, lipids and proteins are also attacked by free radicals induced by ionizing radiation (9, 34, 35). The initiated chain reaction caused by ionizing radiation leads to the formation of a variety of degradation products in biological membranes including products of lipid breakdown. These degradation products induce changes in membrane structure and function that are exaggerated by the accumulation of free radical–mutilated proteins. These degradation products make membranes more rigid (less fluid) and furthermore, after they migrate out of the membrane they react with proteins and nucleic acids, thereby contributing to DNA damage and mutagenesis. Significant changes in structure and function of membranes result in cell death *via* apoptosis (36).

In a lipid-rich environment, as in membranes, both the phospholipid acyl chain and phospholipid backbone as well as cholesterol are subject to radiation damage. However, polyunsaturated hydrocarbon moieties of the phospholipids are particularly sensitive to attack by reactive oxygen species. Exposure of liposomes to γ-radiation confirms the particular susceptibility of polyunsaturated acyl chains to oxidative damage (37). Thus, the radical chain reactions induced by ionizing radiation result in the production of hydroperoxides at the unsaturated sites of lipid acyl chains; hydroperoxide residues change the hydrophobic interactions between adjacent chains of phospholipids allowing easier penetration of water molecules to the most external portion of the bilayer (38), thereby altering the electric constant across the bilayer (39). The altered gradient of water concentrations throughout the membrane has relevant biological consequences since it changes membrane substructure directly and indirectly leads to the degradation of lipids and proteins. Membrane damage caused by ionizing radiation is of particular importance because these altered molecules are not repaired after exposure either to low fractionated doses or to a single acute dose of ionizing radiation (40).

Another commonly measured parameter of lipid damage after ionizing radiation exposure is thiobarbituric acid reactive substances (TBARS). These products, which include malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA), result from the interaction of free radicals with polyunsaturated fatty acids (PUFA). The method for measuring

TBARS, however, has some limitations (41, 42), and the quantity of TBARS measured is highly dependent on the interval after ionizing radiation (41, 43–46). The formation of TBARS as an index of lipid breakdown is a complex process which is due to the high susceptibility of PUFA to free radicals, the production of lipid peroxyl radicals ($LOO^{\bullet}$), endoperoxides and hydroperoxides (47), and, thereafter, the propagation of a chain reaction leading to the extensive lipid damage (48). During this process the fine structure of the biological membranes is modified gradually in different ways and to different extents by end products of lipid peroxidation. Thus, the lack of detection of TBARS in membranes shortly after oxidative stress (e.g., ionizing radiation), and their increasing levels at later intervals is the result of a chain reaction in membranes and the induction of a variety of indirect mechanisms related to the decrease in antioxidant capacity of the membranes.

Another biochemical parameter used as an index of radiation-induced damage to membranes is the level of isoprostanes. In humans, ethane exhaled from the respiratory system is known to be increased after total body irradiation (49). The level of conjugated dienes and ketone dienes, other indices of LPO, also increase in rat liver after exposure to ionizing radiation (45).

The evaluation of oxidative damage to membranes by fluorescence spectroscopic methods is often used because the techniques are both sensitive and reliable (40, 42, 50–52). The motion of spin labels and fluorescence probes intercalated into membranes results from their interaction with both lipids and proteins (53). Two common specific lipophilic probes (i.e., 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene p-toluene sulfonate (TMA-DPH)), are used to measure membrane fluidity (the inverse of membrane rigidity). Of these two probes, TMA-DPH, anchored close to the polar heads of the phospholipid bilayer (the more rigid part of the membrane), seems to be superior to DPH in estimating membrane fluidity. Anisotropy values recorded for TMA-DPH are usually higher, and they usually appear earlier than those for DPH after exposure to ionizing radiation (52). The reliability of membrane fluidity rather than the measurement of TBARS in biological membranes, under certain experimental conditions, results for different reasons. The techniques that use ethidium bromide to reveal changes in membrane permeability show that these alterations begin 6–8 hr after exposure to irradiation (54). The changes in membrane fluidity depend on both the lipid and protein composition of the specific membrane being investigated (55).

Changes in membrane fluidity after exposure to ionizing radiation provides information not only about the lipid bilayer environment but also about changes in the amount of protein damage and the accumulation of water. The latter alteration can change intra- and intermolecular forces that determine the structure of the multilayered membrane, thus influencing the phospholipid heads within the depth of the membrane. As noted above, exposure of biological membranes to ionizing radiation also seriously damages intrinsic proteins. Detectable graded changes in the structure and function of proteins due to the action of reactive oxygen species (56) are reported after increasing doses of ionizing radiation (35, 57, 58). Thus, alterations in the properties of biological membranes caused by ionizing radiation are due to complex processes involving both lipids and proteins. Therefore, changes in membrane fluidity reliably represent the alterations in membrane structure, whereas changes in LPO result only from lipid damage.

Protective Action of Ionizing Radiation Applied in Low Doses. The majority of studies using ionizing radiation are devoted to its negative effects, related especially to genetic damage seen after high radiation doses (4). However, a substantial body of data provides evidence for a protective action of ionizing radiation when applied in low doses (59, 60). Ionizing radiation at low levels brings about what are referred to as adaptive responses and the stimulation of protective physiological mechanisms. These adaptive responses are specific for doses typically below 0.5 Gy (61). The antioxidant properties of ionizing radiation at low doses as well as the protective effects of pretreatment with low-dose ionizing radiation against the damaging effects of high doses have been observed in a variety of studies using different parameters of oxidative damage (e.g., chromosome aberrations and micronuclei formation in human blood lymphocytes (12, 28) and the level of LPO and the activities of the antioxidant enzymes (62–64)). Thus, the effects of low-dose ionizing radiation are similar to those brought about by antioxidants. Interestingly, in a comparative study of ionizing radiation at a 50 cGy dose and the antioxidant melatonin, these factors reveal a similar protective action against chemically induced LPO in mouse brain (64).

Physiological Defense Against Oxidative Stress. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species (65, 66). The antioxidative defense system is composed of methods to (i) transfer sensitive material to compartments better protected from the action of reactive species, (ii) complex transition metals, a potential source of electrons, thereby rendering them unreactive, (iii) inhibit vulnerable processes such as DNA replication, (iv) repair damaged molecules, (v) initiate apoptosis, and, possibly the most important considering the lability to internal and external modifying factors, (vi) activate antioxidant enzymes and finally (vii) use a variety of direct free radical scavengers (65, 66). Of the enzymes involved in antioxidative defense, particularly well documented are the antioxidative properties of the superoxide dismutases (SOD), glutathione peroxidases (GSH-Px), and catalase (CAT) (11).

Since the amount of DNA damage caused by ionizing radiation is correlated with the intensity of oxidative stress (1–3), with the efficacy of defense mechanisms that metabolize toxic intermediates (67) and with intrinsic repair

mechanisms (22), there are several potential means to reduce macromolecular damage due to ionizing radiation. Since radiation-induced cellular DNA damage is primarily attributed to the damaging effects of free radicals, molecules with direct free radical scavenging properties are particularly promising as radioprotectors. The involvement of free radical scavengers in protecting against radiation damage is emphasized by the observation that whole body irradiation decreases the total antioxidant capacity of the organism and depletes the levels of known antioxidants such as ascorbic acid and uric acid as they are used in the scavenging of free radicals (68).

A number of different substances have been examined as to their radioprotective effects against cellular damage caused by ionizing radiation. Several antioxidants have been proven to be efficient protectors against ionizing radiation (e.g., 5-aminosalicylic acid and ascorbate against 8-OHdG formation in calf thymus DNA (24), flavonoids, especially luteolin, against lipid peroxidation, and micronuclei formation in mouse peripheral blood erythrocytes (69) and panthotenol against lipid peroxidation in rat liver (45). These free radical scavengers presumably have protective effects *via* similar actions.

Antioxidative Properties of Melatonin and its Potential Protection Against Carcinogenesis

Melatonin (N-acetyl-5-methoxytryptamine), which was discovered about 40 years ago, is a ubiquitously acting molecule with several functions. It was initially recognized as a molecule related to neuroendocrine physiology, especially reproductive physiology (70, 71). Thereafter, melatonin was found to be involved in the control of circadian rhythms and possibly sleep processes in diurnal species (72, 73). In more recent years, melatonin's ability to influence immune function (74) and cancer growth (75), and its antioxidative properties have been uncovered (76).

Distribution and Mechanisms of Action. Important features of a satisfactory antioxidant are its wide distribution within tissues and cells as well as in subcellular compartments, its ability to cross morphophysiological barriers, and its rapid transport into cells. The apparent ability of melatonin to enter all cells in the organism and all cellular compartments relates to its physical and chemical properties. Melatonin is highly lipid (77) and somewhat aqueous (78) soluble. Binding proteins have been described for melatonin, which may account for the ability of cells to concentrate it against a gradient. Within cell membranes, melatonin may align itself in a position to scavenge free radicals in both the lipid and aqueous environments of the membrane (79, 80).

Within a single cell, melatonin has been found in different compartments (e.g., in the cytosol (81), nucleus (81–83), and cellular membranes (79)); however, highest concentrations of the indole may be presented in the nucleus (82, 83). Thus, melatonin may be in highest concentrations in the portion of the cell (the nucleus) that contains the most sensitive target molecule to ionizing radiation, namely, DNA.

The widespread subcellular distribution of melatonin may allow it to interact with all molecules, thereby reducing oxidative damage to molecules in both the lipid and aqueous environments of the cell. Certainly, this view is supported by the experimental observations showing that melatonin protects lipids in membranes, proteins in the cytosol, and DNA in the nucleus from free radical damage (84).

Clearly, some effects of melatonin are mediated *via* membrane (85–87) and possibly by nuclear receptors (88–91). Its direct free radical scavenging effects, however, are receptor-independent (92, 93). Some of melatonin's indirect antioxidative actions (e.g., stimulation of antioxidative enzymes) may well involve specific receptors for the indole (84).

Both membrane and nuclear receptors for melatonin have been identified. Membrane receptors seem to be widespread but have been most thoroughly investigated in the brain (85) and immune system (86). These receptors are members of the G-protein-linked receptor superfamily (87). Melatonin also influences a number of intracellular events that rely on calcium *via* binding to calmodulin (94). Of recent interest is the identification of nuclear binding sites for melatonin. Melatonin nuclear binding sites were initially described in the liver (88, 89) and subsequently found essentially in all cells (90, 91).

At least in part, the marked protective effects of melatonin against oxidative stress are aided by its ability to cross all biological membranes and the fact that it does not require a receptor for this action. The fact that melatonin diffuses easily and quickly passes through all biological membranes to enter cells and their subcellular compartments is documented (84, 95). The organs of origin of melatonin are still the subject of scientific investigation; however, the pineal gland is generally agreed to be a primary source of this and other indoleamines (84). The highest production of pineal melatonin occurs at night, resulting in high nocturnal concentrations in blood, and consequently, in other bodily fluids as well. In bodily fluids such as the saliva, the nighttime increase in the concentration of melatonin is less than that measured in the blood (84). Whether similar 24-hr fluctuations of melatonin occur within cells remains essentially uninvestigated.

There is some evidence that any external or internal insult that initiates free radical generation in an organism may lead to a reduction in circulating melatonin levels (96–99). This feature of melatonin is especially valuable in case of the exposure to ionizing radiation. Ionizing radiation causes oxidative damage to radiosensitive tissues within an extremely short period, and possible protection against it would require the rapid transfer of an antioxidant to the sensitive cells. At this point, it is not known whether there is a differential uptake of melatonin by various tissues. The presence of melatonin or even its production by radiosensitive tissues such as the retina (100), gastrointestinal tract

(101), ovaries (102), testes (103), and bone marrow (104) suggests that tissues easily damaged by ionizing radiation may either produce or quickly take up melatonin from the blood.

Melatonin as a Free Radical Scavenger. The ability of melatonin to scavenge free radicals is undoubtedly an important property in its protection against oxidative stress. The discovery that melatonin is effective in antioxidative defense is related to the finding that under both *in vitro* and *in vivo* conditions this molecule directly scavenges the highly toxic $^{\bullet}OH$ to form cyclic 3-hydroxymelatonin (3-OHM), a stable metabolite of melatonin (105–112). Melatonin scavenges $^{\bullet}OH$ with a very high rate constant, roughly on the order of $2.7 \times 10^{10}\ M^{-1}\ s^{-1}$ (107). Of particular significance is the fact that the exposure of rats to whole body irradiation increases the level of urinary 3-OHM (113). These levels correlate well with the number $^{\bullet}OH$ generated *in vivo* (110). Moreover, melatonin given prior to whole body irradiation caused a further increase in 3-OHM formation when compared with the effect of the treatment with melatonin only (110). Thus, the metabolite of melatonin, 3-OHM, may be a valuable biomarker of *in vivo* $^{\bullet}OH$ generation. The finding of 3-OHM in human and rat urine under normal physiological conditions (110) provides direct evidence that endogenously produced melatonin scavenges $^{\bullet}OH$ to form its metabolite, 3-OHM. The fact that oxidative damage caused by ionizing radiation is known to be related to the action of $^{\bullet}OH$ (8) makes the finding of Tan *et al.* (105, 110) and other investigators (106–109, 111, 112) particularly important. Interestingly, some methoxylated and hydroxylated indoles, particularly 5-methoxytryptamine (105, 107), 6-chloromelatonin, 5-hydroxytryptamine, 6-hydroxymelatonin (107), and pinoline (6-methoxy-tetrahydro-beta-carboline) (112) also have some ability to scavenge $^{\bullet}OH$.

Besides $^{\bullet}OH$, several other reactive species are also scavenged by melatonin. Compared with $^{\bullet}OH$, these agents are considered to be less toxic and less involved in the damage caused by ionizing radiation. However, several are involved in radical chain reactions that directly or indirectly influence cellular damage. Since ionizing radiation causes lipid damage as well (34, 35, 37–39) and because $LOO^{\bullet}$ propagates the radical chain reaction in the lipid environment, the reported ability of melatonin to scavenge $LOO^{\bullet}$ (114–118) is of understandable interest. One report even claims that melatonin is more effective in scavenging $LOO^{\bullet}$ than is vitamin E (114), which is regarded as a premier lipid antioxidant. Although the superiority of melatonin to vitamin E in scavenging $LOO^{\bullet}$ has not been confirmed (117), the efficacy of melatonin as a lipid antioxidant, regardless of the mechanisms involved, is not questioned (50, 51, 119, 120).

Melatonin has also been shown to quench singlet oxygen ($^1O_2$) (121), a high energy form of O_2 that exhibits high toxicity at the molecular level. Also the peroxynitrite anion ($ONOO^-$), the highly destructive product of the interaction between the superoxide anion radical ($O_2^{-\bullet}$) and nitric oxide ($NO^{\bullet}$), is scavenged by melatonin (122, 123). There is also evidence that melatonin directly neutralizes $NO^{\bullet}$ (124). Melatonin, as it donates an electron in the process of detoxifying electron-deficient reactive oxygen species (92, 93, 106, 107, 125), itself becomes a radical, the melatonyl cation radical; this radical has very low reactivity (92). The melatonyl cation radical may be recycled back to melatonin (126) and theoretically reacts with the $O_2^{-\bullet}$ to produce N^1-acetyl-N^2-formyl-5-methoxykynuramine (92). If melatonin reacts indirectly with $O_2^{-\bullet}$, it would reduce the formation of the hydrogen peroxide (H_2O_2) and, consequently, the formation of $^{\bullet}OH$. Finally, evidence has also accumulated to show that melatonin directly detoxifies H_2O_2 (Tan *et al.*, unpublished data). Since H_2O_2 is the precursor of the $^{\bullet}OH$, its removal reduces formation of the $^{\bullet}OH$.

Effects of Melatonin on the Activities of Enzymes Involved in Antioxidative Defense. The main antioxidant enzymes are SOD, CAT, GSH-Px, glutathione reductase (GSH-Rd), and glucose-6-phosphate dehydrogenase (G6PD). The influence of melatonin on the activities of each of these enzymes showed that the indole stimulated the activity of each of them (84, 119, 127, 128). By increasing the activities of antioxidant enzymes, melatonin reduces the number of free radicals or ROS generated and increases the production of molecules protecting against oxidative stress.

SOD dismutases $O_2^{-\bullet}$ to H_2O_2, decreasing the amount of $O_2^{-\bullet}$ and the formation of $ONOO^-$. Melatonin increases tissue mRNA levels of two isoforms (manganese/copper SOD) of this enzyme (129, 130). Thus, melatonin may decrease the quantity of $O_2^{-\bullet}$ in two ways, directly by stimulating SOD and indirectly when the melatonyl cation radical scavenges it.

Extensive studies have been conducted on melatonin's influence on glutathione (GSH) metabolism. GSH is a well-known antioxidant. Melatonin stimulates the activity of GSH-Px, which transforms H_2O_2 to O_2 (131–135). In this process GSH is oxidized, thus forming oxidized glutathione (GSSG). The reduced form, GSH, is replenished by the action of the enzyme GSH-Rd, the activity of which is also stimulated by melatonin (133, 134). The latter process requires the co-factor NADPH generated from NADP in the reaction catalyzed by G6PD; melatonin also reportedly increases the activity of this enzyme (136). Recently melatonin was also shown to stimulate the activity of γ-glutamylcysteine synthetase (γ-GCS) (137), the rate-limiting enzyme in GSH synthesis. The stimulation of γ-GCS is mediated by activator protein-1 (AP-1), an oxidative stress-responsive transcription factor. The process in question was accompanied by the increased number of cells in the G_0/G_1 phase, which suggests that melatonin, while stimulating GSH synthesis, suppresses cell proliferation (137). In general, the ability of melatonin to increase the metabolism of GSH, an effective antioxidant, is well documented. Finally,

melatonin was recently found to stimulate CAT (138), thereby further reducing H_2O_2 levels and $^{\bullet}OH$ generation.

Besides the antioxidative enzymes mentioned above, the activity of one pro-oxidative enzyme (i.e., nitric oxide synthase (NOS)), is also altered by melatonin. Melatonin has been found to inhibit the activity of NOS (139–141), the enzyme catalyzing the formation of $NO^{\bullet}$. Melatonin, by inhibiting NOS activity, decreases the formation of $NO^{\bullet}$ and the product of its interaction with $O_2^{-\bullet}$, $ONOO^-$. The reported effects of melatonin on the activity of anti- and pro-oxidative enzymes are summarized in Table I.

Melatonin in Physiological Concentrations Acts as an Efficient Antioxidant. The physiological concentrations of radioimmunoassayable melatonin in human blood are roughly 0–20 pg/ml during the day and 40–200 pg/ml during the night (142). These serum levels are lower than those measured after its exogenous administration. However, studies devoted to antioxidative properties of melatonin have provided evidence that endogenously produced melatonin is in sufficient amounts to protect against oxidative stress. For example, melatonin in physiological doses reduced DNA adduct formation induced by the carcinogen safrole (143), protected against brain damage related to stroke or excitotoxic seizures (144), and prevented lethal oxidative stress in *Gonyaulax polyedra (145).*

In aging studies in rats, Reiter *et al.* (146) observed much higher 8-OHdG and MDA+4-HDA levels and increased microsomal membrane rigidity (indices of oxidative damage, which are also caused by ionizing radiation) in a variety of organs collected from 25-month-old rats when compared with 2-month-old controls; when the rats were pinealectomized at the age of 2 months, which caused a relative melatonin deficiency, this brought about a further increase in these parameters of oxidative damage when measured in rats at the age of 25 months. Since both aging and pinealectomy (147–149) led to a reduction in pineal-derived melatonin, it is suggested that a reduction in physiological levels of this indoleamine may contribute to the increase in oxidative damage in advanced age.

That endogenously produced melatonin contributes to the total antioxidant status of the organism has been documented. In both rats and humans, the total antioxidative capacity of the serum correlated with the levels of melatonin (150, 151). Thus, they exhibit a synchronous circadian rhythm with nocturnal peaks and day-time troughs (150, 151). Nocturnal exposure to light or treatment with antibodies against melatonin brought about a reduction of nocturnal peak of the total antioxidant status (151). Furthermore, the total antioxidant status, like melatonin, exhibited a more pronounced circadian rhythm in young individuals, and an aging-dependent decreasing night-day amplitude (151). Similarly, a correlation exists between the circadian rhythm of melatonin and phagocytosis in ring dove heterophils; at the same time a higher level of $O_2^{-\bullet}$ production by phagocytes occurred during the light period and a lower level during the day (152).

The implication of these studies is that melatonin at physiological levels contributes to the total antioxidant status of organisms. Likewise, it is well documented that supplementing animals with melatonin increases the capacity of the organism to resist oxidative damage.

Other Properties of Melatonin Confirming its Potential Antioxidant Capacity in Protection Against Damage Caused by Ionizing Radiation. The contribution of the immune system to defense against oxidative stress is probably not a direct one. However, the efficiency of the immune system improves the function of other systems and mechanisms in living organisms. The connection between melatonin and the neuroimmunoendocrine system is well known (74) and, additionally, a connection between the immune system and antioxidant status was recently suggested (152).

Melatonin has been reported to protect against apoptosis in rat neurons *in vitro* (121), in rat thymocytes *in vivo* and *in vitro* (153), and mouse bone marrow cells *in vivo* and *in vitro* (154). In view of the protective action of melatonin against apoptosis and radiation-induced diurnal changes in apoptosis (155), a preventive effect of melatonin against apoptosis caused by ionizing radiation seems probable, but requires experimental proof.

Melatonin as an Antioxidant and Free Radical Scavenger in Defense Against Different Carcinogens—Examples. The literature related to the protective role of melatonin against oxidative damage caused by carcinogens, other than ionizing radiation, is abundant. The indoleamine highly efficiently protects against damage of all macromolecules (i.e., DNA (108, 143, 156–161), lipids (127, 128, 162–164) and proteins (165). The protection of nuclear DNA from reactants that generate free radicals and duplicate the effects of ionizing radiation (i.e., from ionizing radiation mimics) are especially noteworthy (166).

Table I. Reported Effects of Melatonin on the Activities of Anti- and Pro-Oxidative Enzymes

Enzymes	Potential changes resulting from the action of melatonin	Ref
⇑ Superoxide dismutase	⇓ $O_2^{-\bullet}$	129,130
⇑ Catalase	⇓ H_2O_2	109
⇑ Glutathione peroxidase	⇓ H_2O_2	131–135
⇑ Glutathione reductase	⇑ GSH	133,134
⇑ γ-Glutamylcysteine synthetase	⇑ GSH	137
⇑ Glucose-6-phosphate dehydrogenase	⇑ NADPH	136
⇓ Nitric oxide synthase	⇓ $NO^{\bullet}$, ⇓ $ONOO^-$	139–141

Note. ⇓ = decrease; ⇑ = increase; $O_2^{-\bullet}$ = superoxide anion radical; H_2O_2 = hydrogen peroxide; GSH = glutathione; $NO^{\bullet}$ = nitric oxide; $ONOO^-$ = peroxynitrite anion.

Melatonin Counteracts Carcinogenic Action of Ionizing Radiation by Antioxidative Mechanisms—Experimental Evidence

Studies on the protective effects of melatonin against damage caused by ionizing radiation began soon after the discovery of its antioxidative properties. Actually, the first study that revealed the antioxidative effects of melatonin to a physical agent was conducted using ultraviolet light (UV), which shares many features with ionizing radiation. In 1993, Tan *et al.* (105) reported that melatonin scavenges $^{\bullet}OH$ generated *in vitro* when H_2O_2 was exposed to ultraviolet light. *In vivo* as well, UV light damage to the skin is known to be prevented by melatonin. Using a similar model, the potency of melatonin in protecting against $^{\bullet}OH$ generated by ionizing radiation was confirmed under *in vivo* conditions (110). In the meantime, a number of studies have tested melatonin's efficacy in protecting against ionizing radiation both *in vivo* and *in vitro*.

***In Vivo* Studies in Animals.** Already in 1994, Blickenstaff *et al.* (167) carried out studies illustrating the radioprotective actions of melatonin in Swiss ND4 mice. They noted that their exposure to 950 cGy of whole body radiation caused the death of all animals 12 days later, whereas when mice were pretreated with melatonin (1.076 mmol/kg body wt), 43% of the irradiated mice survived at least 30 days after treatment. Interestingly, homologs of melatonin obtained by acylation of 5-methoxytryptamine revealed protective effects against ionizing radiation as well; maximal survival rate was achieved with homologs having acyl chain lengths of six or eight carbons.

A similar survival study was conducted by Vijayalaxmi *et al.* (168) using CD2-F1 mice. They observed that the exposure of mice to 815 cGy of ionizing radiation resulted in only a 45%–50% survival rate after 30 days; pretreatment with melatonin at a dose of 125 mg/kg body wt slightly increased the survival rate to 60%, whereas melatonin at a dose of 250 mg/kg body wt significantly increased survival to 85%.

Other studies have examined lipid damage after ionizing radiation and the protective effects of melatonin. Exposure of Wistar female rats to ionizing radiation at two doses of 360 cGy radiation given in an interval of 12 hr, brought about, expectedly, significant increases in the levels of MDA in ovaries and plasma 6 hr after radiation exposure (169). Pretreatment of the rats with melatonin at a dose of 100 mg/kg body wt decreased the level of MDA in both tissues when compared with that in irradiated animals. A study performed on Wistar:Han SPF rats has found that melatonin administered in drinking water (dose not specified) for 15 days prevented the increase in hepatic lipids and thymic triacylglycerol levels caused by continuous irradiation at a daily dose of 96 mGy and/or dimethylbenz(a)anthracene treatment, also given daily for 15 days (170).

Experiments have also been performed to examine the early effect of ionizing radiation on both *in vivo* DNA and membrane damage in rats. Whole body irradiation at a dose of 800 cGy to Sprague-Dawley male rats increased oxidation of guanine bases in DNA isolated from liver collected 12 hr later (Karbownik *et al.*, unpublished data). Similarly, radiation exposure decreased membrane fluidity of hepatic microsomes, although it did not influence lipid breakdown as evaluated by the level of MDA+4-HDA in liver and other tissues. These early effects of ionizing radiation on DNA damage and membrane fluidity were, expectedly, prevented by melatonin injected at a dose of 50 mg/kg body wt at 120, 90, 60, and 30 min prior to radiation exposure.

Another study performed by Vijayalaxmi *et al.* (171), using CD2-F1 male mice, revealed a protective effect against ionizing radiation-induced damage to the genome of hematopoietic cells. The exposure of mice to whole body irradiation at a dose of 150 cGy resulted in a reduced percentage of polychromatic erythrocytes in the peripheral blood and bone marrow cells and an increased incidence of micronuclei in the same cells measured 24 hr later. However, melatonin, when injected 1 hr prior to irradiation, in doses of 5 mg/kg body wt or 10 mg/kg body wt, partially reversed the damaging effect of radiation with the higher dose being more effective.

A similar study to one described above was performed using mice. Whole body irradiation at a dose of 1.5 Gy brought about a marked increase in the frequency of micronuclei in polychromatic erythrocytes and of chromosomal aberrations in spermatogonia and spermatocytes 24 hr later (172). Melatonin given at a dose of 10 mg/kg body wt, 1 hr prior to irradiation, significantly decreased the effects of this exposure. The authors of the present survey underline that they do not entirely agree with the interpretation of the results in the report by Badr *et al.* (172). Their conclusion related to "a cytotoxic effect of melatonin at a relatively high dose (10 mg/kg body wt)" (172) is not supported, in the opinion of the authors of the present survey, by either their findings (172) or other literature. The very small increase in micronuclei in polychromatic erythrocytes after a 10 mg/kg body wt dose of melatonin, when compared with the effect of ionizing radiation, would seem irrelevant. Worth underlining is the fact that melatonin did not influence any other examined parameter of chromosomal damage and, when applied before exposure to radiation, decreased the damaging effect of ionizing radiation in all measured parameters, micronuclei included (172). These *in vivo* studies are summarized in Table II.

Studies Using Human Tissues *In Vitro*. Investigations regarding the influence of ionizing radiation on humans have obvious limitations. The initial *in vitro* studies that used human tissues to test the protective effects of melatonin against ionizing radiation-induced damage were done by Vijayalaxmi *et al.* (29, 30). The authors used human peripheral blood lymphocytes that are not only easy to obtain but also highly radiosensitive, and both ionizing radiation and melatonin were applied to cultured cells. Cells exposed to γ-irradiation at a dose of 1.5 Gy exhibited a

Table II. Studies in Animals Supporting the Protective Effects of Melatonin Against Oxidative Damage Caused by Ionizing Radiation

Species	Dose of ionizing radiation	Dose of melatonin, time before radiation	Measured parameter/effect of melatonin in irradiated animals	Ref
Swiss ND4 mice	950 cGy	250 mg/kg b.w.	percentage of mice surviving 30 days after irradiation: 43% when compared with 0% in mice irradiated only	167
Wistar:Han SPF rats	96 mGy daily within 15 days	in drinking water during the exposure to radiation (dose not specified)	⇓ oxidized lipid level in the liver and triacylglycerols in the thymus	170
CD2-F1 mice	815 cGy	250 mg/kg b.w., 1 hr	percentage of mice surviving 30 days after irradiation: 85% when compared with 45%–50% in mice irradiated only	168
CD2-F1 mice	150 cGy	10 mg/kg b.w., 1 hr	⇑ percentage of polychromatic erythrocytes in the peripheral blood and bone marrow cells; ⇓ incidence of micronuclei in the polychromatic erythrocytes in the peripheral blood and bone marrow cells	171
Albino mouse bone marrow	150 cGy	10 mg/kg b.w., 1 hr	⇓ frequency of micronuclei in polychromatic erythrocytes; ⇓ chromosomal aberrations in spermatogonia and spermatocytes	172
Wistar rats	2 doses of 360 cGy separated by 12 hr	100 mg/kg b.w.	⇓ MDA in ovary and blood plasma	169
Sprague-Dawley rats	800 cGy	200 mg/kg b.w.	⇓ 8-OHdG in liver; ⇑ membrane fluidity in liver	[a]

Note. ⇓ = decrease; ⇑ = increase; MDA = malondialdehyde; 8-OHdG = 8-hydroxy-2′-deoxyguanosine.
[a] Karbownik *et al.*, unpublished data.

pronounced increase in chromosome damage (29); this was verified by the increased incidence in abnormal cells, an augmentation of exchange-type aberrations, and a rise in the frequency of acentric fragments. Preincubation (20 min) with melatonin at concentrations of either 0.5, 1.0, or 2.0 m*M* reduced concentration dependently the incidence of abnormal cells by 31.3%, 47.8%, and 61.9%, the incidence of exchange chromosomes by 34.3%, 53.3%, and 68.0%, and the frequency of acentric fragments by 42.4%, 53.5%, and 67.8%, respectively.

Using similar experimental conditions, an additional study by Vijayalaxmi *et al.* (30) revealed a significant increase in micronuclei formation after exposure of cells to γ-irradiation at a dose of 1.5 Gy. In this study there was both an increased number of cytokinesis-blocked binucleate cells with micronuclei and an increased total number of micronuclei. Preincubation (20 min) with melatonin at concentrations of either 0.5, 1.0, or 2.0 m*M* reduced, in a concentration-dependent manner, the number of binucleate cells by 32.5%, 45.4%, and 61.6% and the total number of

Table III. Studies *In Vitro* Providing Evidence of the Protective Effects of Melatonin Against Oxidative Damage Caused by Ionizing Radiation

Cell type	Dose of inonizing radiation	Concentration of melatonin	Measured parameter	Effect	Ref
Human blood lymphocytes	150 cGy	0.5, 1.0, or 2.0 m*M*	Incidence of chromosome aberrations	Concentration-dependent reduction	29
Human blood lymphocytes	150 cGy	0.5, 1.0, or 2.0 m*M*	Frequency of micronuclei formation	Concentration-dependent reduction	30
HeLa S3 cells	50 cGy	0.01 m*M*	DNA binding activity of transcriptional regulator, NF-κB	Decreased	173

Note. NF-κB = nuclear factor-κB.

micronuclei by 36.0%, 50.0%, and 63.7%, respectively. These *in vitro* studies are summarized in Table III.

Studies on Human Tissues When Applying Melatonin *In Vivo*. Satisfactory protective effects of melatonin against ionizing radiation *in vitro* (29, 30) led to subsequent investigations where melatonin was administered *in vivo*. Vijayalaxmi *et al.* (31, 32) examined different parameters of DNA damage in peripheral blood leukocytes obtained before and at 1 and 2 hr following the oral ingestion of melatonin at the dose of 300 mg. Immediately after collecting the blood, the samples were exposed to 150 cGy γ-radiation and then mitogenically stimulated and cultured for 48 or 72 hr. Similar to the results described above (29, 30), exposure to ionizing radiation caused a significant increase in chromosomal aberrations and micronuclei formation (31). Expectedly, the ingestion of melatonin decreased the incidence of both end points with the most pronounced protective effect seen when the cells were examined 2 hr after taking melatonin. Namely, the incidence of abnormal cells with chromosome damage was reduced by 53.6% and 59.8%, the frequency of exchange aberrations was lowered by 56.7% and 61.5%, and the incidence of acentric fragments by 55.9% and 62.7% at 1 and 2 hr after ingestion of melatonin, respectively. Similarly, the number of cells with micronuclei decreased by 57.9% and 63.0% and the total number of micronuclei by 55.9% and 62.7%, respectively.

Additionally, on the same population of blood lymphocytes under similar experimental conditions but using a lower dose of ionizing radiation (100 cGy), Vijayalaxmi *et al.* (32) examined the extent of primary DNA damage determined by the length of DNA migration and the fluorescence intensity of the nuclear tail using the alkaline Comet assay. Ingestion of melatonin reduced γ-radiation-induced increases in lengths of DNA migration by 15.6% and 20.9% after 1 and 2 hr, respectively. Also, the fluorescence intensity measured in the Comet tail decreased by 12.9%–20.5% at 1 hr and by 18.5%–24.0% at 2 hr as the result of melatonin treatment. In this study, an inverse correlation between serum melatonin concentrations and the number of DNA-damaged cells was apparent. These results are summarized in Table IV.

Other Evidence of Protective Effects of Melatonin Against Damage Caused by Ionizing Radiation. The activation of an important transcriptional regulator, nuclear factor-κB (NF-κB), requires free radicals as second messengers. This factor transactivates many genes involved in immune, inflammatory, and acute-phase responses, as well as genes related to cell proliferation and differentiation. Marked increases in NF-κB binding activity has been observed in HeLa S3 cells as the result of their exposure to ionizing radiation at a dose of 0.5 Gy (173). This process was partially reversed by melatonin. The preincubation with the indoleamine at a concentration of 0.01 m*M* within 30 min caused a 29.3% decrease in NF-κB expression. Thus, melatonin influences physiological and pathological processes by modulating the expression of NF-κB and possibly other transcriptional regulators.

There is some evidence for the protective role of melatonin against radiation-induced damage in the endocrine system. It was observed that pretreatment with melatonin partially reversed histoenzymological changes in rat thyroid gland caused by the exposure to 8 Gy of γ-radiation (174). In another study that used morphometric parameters, melatonin was shown to decrease the height of the thyroid follicular cells and nuclear volume of these cells obtained from 8 Gy-irradiated rats (175). Melatonin treatment also inhibited the effect of γ-irradiation at a dose of 8 Gy on several morphometric characteristics of the seminiferous tubules in rats (176). Similarly, the treatment of rats for 2 weeks with melatonin at a daily dose of 0.2 mg significantly decreased what the authors referred to as the nuclear and cytoplasm surface of Leydig cells in rats exposed to 8 Gy radiation (177).

Toxicology of Melatonin. Melatonin has been tested over a wide range of doses from physiological to high pharmacological concentrations in different species as to its potential toxicology. There is little evidence at this point to suggest major unfavorable effects of melatonin. The usual doses given *in vivo* are as follows: 10 mg/kg body wt in mice (171), 100 mg/kg body wt in rats (169), 200 mg/kg body wt in rats (Karbownik *et al.*, unpublished data), 250 mg/kg body wt in mice (167, 168), 250 mg/kg in pregnant rats (178), or even 800 mg/kg body wt in mice, rabbits, cats, and dogs (179). Recently, no toxicity of melatonin to mouse embryos was observed when it was given during pregnancy at doses up to 200 mg/kg body wt (180). Particularly worth underlining are studies on human volunteers, in which no adverse side effects have been observed after oral administration of melatonin in doses of 1–300 mg (31, 32, 181–187) and 1 gram of melatonin daily for 30 days (188).

Table IV. Studies in Humans Showing the Protective Effects of Melatonin Applied *In Vitro* Against Oxidative Damage Caused by Ionizing Radiation Applied *In Vitro*

Tissue type	Dose of melatonin *in vivo*	Dose of ionizing radiation *in vitro*	Measured parameter/ effect of melatonin	Ref
Human blood lymphocytes	300 mg orally	150 cGy	⇓ chromosome aberrations and micronuclei	31
Human blood lymphocytes	300 mg orally	100 cGy	⇓ extent of primary DNA damage	32

Note. ⇓ = decrease.

Prooxidative effects of melatonin used in supraphamacological doses have been described only in a few situations, namely in isolated red blood cells (189) and isolated retinal photoreceptors (190). It is important to note these findings, but their significance to the *in vivo* situation remains unknown.

Concluding Remarks

The mechanisms of the radioprotective effects of melatonin seem to consist particularly of its scavenging $^{\bullet}OH$ and other free radicals and reactive oxygen intermediates, and its ability to modify the activity of enzymes participating in oxidative stress. Besides the obvious antioxidative mechanisms by which melatonin functions in the protection of DNA, it also may influence DNA repair enzymes directly and stimulate intracellular signals indirectly for the activation of genes responsible for protein synthesis related to DNA repair. These latter processes deserve more extensive investigation in relation to melatonin.

A decreased total antioxidant capacity of plasma of patients exposed to whole body irradiation for the purpose of reducing tumor growth or because of its immunosuppressive effect has been reported (68). Since the total antioxidant capacity of human serum positively correlates with its melatonin content (150), supplementing patients, who are exposed therapeutically or diagnostically to ionizing radiation, with melatonin may have some benefit.

On the basis of the evidence described in the present survey, the following features make melatonin a potentially useful radioprotector: (i) direct and indirect scavenging of free radicals (105–112, 114–124); (ii) the ability to stimulate the activity of antioxidant enzymes (109, 131–137) and to inhibit the activity of a pro-oxidative enzyme (139–141); (iii) distribution in all tissues, cells, and cellular compartments throughout the organism (84, 95); (iv) quick diffusion through all biological membranes (84, 95); (v) ability to provide radioprotection without receptor interaction (92, 93); (vi) presence in radiosensitive tissues (100–104); (vii) tolerance to high doses of melatonin by living organisms and little toxicity in the species tested (31, 32, 167–171, 178–188); (viii) antioxidant efficacy in physiological concentrations (143–146, 150–152); (ix) correlation of melatonin concentration with total antioxidant activity (150, 151); (x) antioxidative protection against all macromolecules (i.e., DNA (29–33, 108, 143, 156–161, 171), lipids (127, 128, 162–164) and proteins (169); (xi) ease of oral administration; and (xii) high bioavailability when administered by virtually any route.

The evidence summarized herein, related to radioprotective effects of melatonin against cellular damage caused by oxidative stress, make this molecule a potential supplement that should be considered in the treatment or cotreatment in situations where the effects of ionizing radiation are to be minimized (e.g., during routine therapies, in occupational settings where accidental exposure may occur, during space travel, or after nuclear accidents, and where high radiation persists).

1. Toule R. Radiation-induced DNA damage and its repair. Int J Radiat Biol **51**:573–589, 1987.
2. Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. Mutat Res **250**:3–16, 1991.
3. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. FEBS Lett **281**:9–19, 1991.
4. Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL. Genomic instability induced by ionizing radiation. Radiat Res **146**:247–258, 1996.
5. Becker D, Sevilla MD. The chemical consequences of radiation damage to DNA. Adv Radiat Biol **17**:121–179, 1993.
6. Ikushima T, Aritomi H, Morisita J. Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells. Mutat Res **358**:193–198, 1996.
7. Wallace SS. Enzymatic processing of radiation-induced free radical damage in DNA. Radiat Res **150**:S60–S79, 1998.
8. Ward JF. DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation, and reparability. Prog Nucl Acid Res Mol Biol **35**:95–125, 1988.
9. Martinez-Cayuela M. Oxygen free radicals and human disease. Biochimie **77**:147–161, 1955.
10. Sies H. Biochemistry of oxidant stress. Angew Chem Int Ed Engl **25**:1058–1071, 1986.
11. De Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med **26**:202–226, 1999.
12. Vijayalaxmi Leal BZ, Deahl TS, Meltz ML. Variability in adaptive response to low dose radiation in human blood lymphocytes: Consistent results from chromosome aberrations and micronuclei. Mutat Res **348**:45–50, 1995.
13. Steenken S. Purine bases, nucleosides, and nucleotides: Aqueous solution redox chemistry and transformation reactions of their radical cations and $e^{\bullet-}$ and OH adducts. Chem Rev **89**:503–520, 1989.
14. Breen AP, Murphy JA. Reactions of oxyl radicals with DNA. Free Radic Biol Med **18**:1033–1077, 1995.
15. Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem **59**:1609–1623, 1992.
16. Oshino N, Chance B, Sies H, Bücher T. The role of H_2O_2 generation in perfused rat liver and the reaction of catalase compound I and hydrogen donors. Arch Biochem Biophys **154**:117–131, 1993.
17. Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL, Sauvaigo S. Hydroxyl radicals and DNA base damage. Mutat Res **424**:9–21, 1999.
18. Kasai H, Crain F, Kuchino Y, Nishimura N, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis **7**:1849–1851, 1986.
19. Gajewski E, Rao G, Nackerdien Z, Dizdaroglu M. Modification of DNA bases in mammalian chromatin by radiation-generated free radicals. Biochemistry **29**:7876–7882, 1990.
20. Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. Mechanistic studies of ionizing radiation and oxidative mutagenesis: Genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry **29**:7024–7032, 1990.
21. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and andA→X substitutions. J Biol Chem **267**:166–172, 1992.
22. Tchou J, Grollman AP. Repair of DNA containing the oxidatively damaged base, 8-oxoguanine. Mutat Res **299**:277–287, 1993.
23. Floyd RA. The role of 8-hydroxydeoxyguanosine in carcinogenesis. Carcinogenesis **11**:1447–1450, 1990.
24. Fischer-Nielsen A, Jeding IB, Loft S. Radiation-induced formation of 8-hydroxy-2'-deoxyguanosine and its prevention by scavengers. Carcinogenesis **15**:1609–1612, 1994.
25. Haegele AD, Wolfe P, Thompson HJ. X-radiation induces 8-hydroxy-2'-deoxyguanosine formation *in vivo* in rat mammary gland DNA. Carcinogenesis **19**:1319–1321, 1998.

26. Elia MC, DeLuca JG, Bradley MO. Significance and measurement of DNA double strand breaks in mammalian cells. Pharmacol Ther **51**:291–327, 1991.
27. Sankaranarayanan K. Ionizing radiation and genetic risks X. The potential "disease phenotypes" of radiation-induced genetic damage in humans: Perspectives from human molecular biology and radiation genetics. Mutat Res **429**:45–83, 1999.
28. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science **223**:594–597, 1984.
29. Vijayalaxmi Reiter RJ, Meltz ML. Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. Mutat Res **346**:23–31, 1995.
30. Vijayalaxmi Reiter RJ, Sewerynek E, Poeggeler B, Leal BZ, Meltz ML. Marked reduction of radiation-induced micronuclei in human blood lymphocytes pre-treated with melatonin. Radiat Res **143**:102–106, 1995.
31. Vijayalaxmi Reiter RJ, Herman TS, Meltz ML. Melatonin and radioprotection from genetic damage: *In vivo/in vitro* studies with human volunteers. Mutat Res **371**:221–228, 1996.
32. Vijayalaxmi Reiter RJ, Herman TS, Meltz ML. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. Mutat Res **397**:203–208, 1998.
33. Vijayalaxmi Reiter RJ, Meltz ML, Herman TS. Melatonin: Possible mechanisms involved in its 'radioprotective' effect. Mutat Res **404**:187–189, 1998.
34. Edwards JC, Chapman D, Cramp WA, Yatvin MB. The effects of ionizing radiation on biomembrane structure and function. Prog Biophys Mol Biol **43**:71–93, 1984.
35. Verma SP, Sonwalker N. Structural changes in plasma membranes prepared from irradiated Chinese hamster V79 cells as revealed by Raman spectroscopy. Radiat Res **126**:27–35, 1991.
36. Lizard G, Fournel S, Genestier L, Dhedin N, Chaput C, Flacher M, Mutin M, Panaye G, Revillard JP. Kinetics of plasma membrane and mitochondrial alterations in cells undergoing apoptosis. Cytometry **21**:275–283, 1995.
37. Samuni AM, Barenholz Y, Crommelin DJA, Zuidam NJ. γ-Irradiation damage to liposomes differing in composition and their protection by nitroxides. Free Radic Biol Med **23**:972–979, 1997.
38. Parasassi T, Giusti AM, Gratton E, Monaco E, Raimondi M, Ravagnan G, Sapora O. Evidence for an increase in water concentration in bilayers after oxidative damage of phospholipids induced by ionizing radiation. Int J Radiat Biol **65**:329–334, 1994.
39. Parasassi T, Ravagnan G, Sapora O, Gratton E. Membrane oxidative damage induced by ionizing radiation detected by diphenylhexatriene fluorescence lifetime distributions. Int J Radiat Biol **61**:791–796, 1992.
40. Giusti AM, Raimondi M, Ravagnan G, Sapora O, Parasassi T. Human cell membrane oxidative damage induced by single and fractionated doses of ionizing radiation: A fluorescence spectroscopy study. Int J Radiat Biol **74**:595–605, 1998.
41. Chatterjee SN, Agarwal S. Liposomes as membrane model for study of lipid peroxidation. Free Radic Biol Med **4**:51–72, 1988.
42. Parasassi T, Sapora O, Giusti AM, De Stasio G, Ravagnan G. Alteration in erythrocyte membrane lipids induced by low doses of ionizing radiation as revealed by 1,6-diphenyl-1, 3,5–hexatriene fluorescence lifetime. Int J Radiat Biol **59**:59–69, 1991.
43. Ueda T, Toyoshima Y, Kushihahi T, Hishida T, Yasuhara H. Effect of dimethyl sulfoxide pretreatment on activities of lipid peroxide formation, superoxide dismutase, and glutathione peroxidase in the mouse liver after whole-body irradiation. J Toxicol Sci **18**:239–244, 1993.
44. Ueda T, Toyoshima Y, Moritani T, Ri K, Otsuki N, Kushihashi T, Yasuhara H, Hishida T. Protective effect of dipyridamole against lethality and lipid peroxidation in liver and spleen of the ddY mouse after whole-body irradiation. Int J Radiat Biol **69**:199–204, 1996.
45. Slyshenkov VS, Omelyanchik SN, Moiseenok AG, Trebukhina RV, Wojtczak L. Pantothenol protects rats against some deleterious effects of gamma radiation. Free Radic Biol Med **24**:894–899, 1998.
46. Ono SI, Cai L, Cherian MG. Effects of gamma radiation on levels of brain metallothionein and lipid peroxidation in transgenic mice. Radiat Res **150**:52–57, 1998.
47. Curtis MT, Gilfor D, Farber JL. Lipid peroxidation increases the molecular order of microsomal membranes. Arch Biochem Biophys **235**:644–649, 1984.
48. Maiorino M, Coassin M, Roveri A, Ursini F. Microsomal lipid peroxidation: Effect of vitamin E and its functional interaction with phospholipid hydroperoxide glutathione peroxidase. Lipids **24**:721–726, 1989.
49. Artebry VE, Pryor WA, Jiang L, Sehnert SS, Foster WM, Abrams RA, Williams JR, Wharam MD, Risby TH. Breath ethane generation during clinical total body irradiation as a marker of oxygen-free-radical-mediated lipid peroxidation: A case study. Free Radic Biol Med **17**:569–576, 1994.
50. Garcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, Munoz-Hoyos A. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. FEBS Lett **408**:297–300, 1997.
51. Garcia JJ, Reiter RJ, Ortiz GG, Oh CS, Tang L, Yu BP, Escames G. Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. J Membr Biol **162**:59–65, 1998.
52. Berroud A, Le Roy A, Voisin P. Membrane oxidative damage induced by ionizing radiation detected by fluorescence polarization. Radiat Environ Biophys **35**:289–295, 1996.
53. Marsh D. Spin-label answers to lipid-protein interactions. Trends Biochem Sci **8**:330–333, 1983.
54. Afanasyev VN, Korol BA, Matylevich NP, Pechatnikov VA, Umansky SR. The use of flow cytometry for the investigation of cell death. Cytometry **14**:603–609, 1993.
55. Zimmer G. Fluidity of cell membranes in the presence of some drugs and inhibitors. In: Kates M, Manson LA, Eds. Biomembranes. New York: Plenum, pp169–203, 1984.
56. Pohl LR. An immunochemical approach of identifying and characterizing protein targets of toxic reactive metabolites. Chem Res Toxicol **6**:786–793, 1993.
57. Le Maire M, Thauvette L, De Foresta B, Viel A, Beauregard G, Poitier M. Effects of ionizing radiation on proteins: Evidence of nonrandom fragmentation and caution in the use of the method for determination of molecular mass. Biochem J **267**:431–439, 1990.
58. Jhun E, Jhun BH, Jones LR, Jung CY. Direct effects of ionizing radiation on integral membrane proteins. J Biol Chem **266**:9403–9407, 1991.
59. Wolff S. Aspects of the adaptive response to very low doses of radiation and other agents. Mutat Res **358**:135–142, 1996.
60. Wolff S. The adaptive response in radiobiology: Evolving insights and implications. Environ Health Perspect **106**:277–283, 1998.
61. Feinendegen LE. The role of adaptive responses following exposure to ionizing radiation. Hum Exp Toxicol **18**:426–432, 1999.
62. Zhang H, Zheng RL, Wei ZQ, Li WJ, Gao QX, Chen WQ, Wang ZH, He J, Liang JP, Han GW, Huang T, Li Q, Xie HM, Zhang SM, Cai XC. Effects of pre-exposure of mouse testis with low-dose $^{16}O^{8+}$ ions or ^{60}Co γ-rays on sperm shape abnormalities, lipid peroxidation, and superoxide dismutase (SOD) activity induced by subsequent high-dose irradiation. Int J Radiat Biol **73**:163–167, 1998.
63. Kojima S, Matsuki O, Nomura T, Kubodera A, Honda Y, Honda S, Tanooka H, Wakasugi H, Yamaoka K. Induction of mRNAs for glutathione synthesis-related proteins in mouse liver by low doses of γ-rays. Biochim Biophys Acta **1381**:312–318, 1998.
64. Kojima S, Matsuki O, Nomura T, Yamaoka K, Takahashi M, Niki E. Elevation of antioxidant potency in the brain of mice by low-dose γ-ray irradiation and its effect on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain damage. Free Radic Biol Med **26**:388–395, 1999.
65. Riley PA. Free radicals in biology: Oxidative stress and the effects of ionizing radiation. Int J Radiat Biol **65**:27–33, 1994.
66. Jaeschke H. Mechanisms of oxidant stress-induced acute tissue injury. Proc Soc Exp Biol Med **209**:104–111, 1995.
67. Sies H. Strategies of antioxidant defense. Eur J Biochem **215**:213–219, 1993.
68. Chevion S, Or R, Berry EM. The antioxidant status of patients subjected to total body irradiation. Biochem Mol Biol Int **47**:1019–1027, 1999.
69. Shimoi K, Masuda S, Furugori M, Esaki S, Kinae N. Radioprotective effect of antioxidative flavonoids in gamma-ray irradiated mice. Carcinogenesis **15**:2669–2672, 1994.

70. Reiter RJ. Comparative physiology: Pineal gland. Ann Rev Physiol **35**:305–328, 1973.
71. Reiter RJ. The pineal and its hormones in the control of reproduction in mammals. Endocr Rev **1**:109–131, 1980.
72. Arendt J. Melatonin. Clin Endocrinol **29**: 205–229, 1988.
73. Reiter RJ. Melatonin: The chemical expression of darkness. Mol Cell Endocrinol **79**:C153–C158, 1991.
74. Maestroni GJM. The immunoendocrine role of melatonin. J Pineal Res **14**:121–126, 1993.
75. Blask DE. Melatonin in oncology. In: Yu HS, Reiter RJ, Eds. Melatonin: Biosynthesis, Physiological Effects, and Clinical Applications. Boca Raton, FL: CRC Press, pp447–475, 1993.
76. Reiter RJ. The pineal gland and melatonin in relation to aging: A summary of the theories and of the data. Exp Gerontol **30**:199–212, 1995.
77. Costa EJX, Lopes RH, Lamy-Freund MT. Solubility of pure bilayers to melatonin. J Pineal Res **19**:123–126, 1995.
78. Shida CS, Castrucci AML, Lamy-Freund MT. High melatonin solubility in aqueous media. J Pineal Res **16**:198–201, 1994.
79. Costa EJX, Shida CS, Biaggi MH, Ito AS, Lamy-Freund MT. How melatonin interacts with lipid bilayers: A study by fluorescence and ESR spectroscopies. FEBS Lett **416**:103–106, 1997.
80. Ceraulo L, Ferrugia M, Tesoriere L, Segreto S, Livrea MA, Liveri TV. Interactions of melatonin with membrane models: Portioning of melatonin in AOT and lecithin reversed micelles. J Pineal Res **26**:108–112, 1999.
81. Menendez-Pelaez A, Reiter RJ. Distribution of melatonin in mammalian tissues: The relative importance of cytosolic versus nuclear localization. J Pineal Res **15**:59–69, 1993.
82. Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden LR, Pablos MI, Tan DX. Nuclear localization of melatonin in different mammalian tissues: Immunocytochemical and radioimmunoassay evidence. J Cell Biochem **53**:372–382, 1993.
83. Finnocchiaro LME, Glikin GG. Modulation of subcellular distribution of calmodulin by melatonin in MDCK cells. J Pineal Res **24**:22–34, 1998.
84. Reiter RJ, Guerrero JM, Garcia JJ, Acuna-Castroviejo D. Reactive oxygen intermediates, molecular damage and aging: Relation to melatonin. Ann N Y Acad Sci **854**:410–424, 1998.
85. Morgan JP. Molecular signaling *via* the melatonin receptor. Adv Pineal Res **5**:191–198, 1991.
86. Poon AMS, Pang SF. Modulation of 2[^{125}I]iodomelatonin binding sites in the guinea pig spleen by melatonin injection is dependent on the dose and period but not the time. Life Sci **54**:1411–1418, 1994.
87. Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron **13**:1177–1185, 1994.
88. Acuna-Castroviejo D, Pablos MI, Menendez-Pelaez A, Reiter RJ. Melatonin receptors in purified cell nuclei of liver. Res Commun Chem Pathol Pharmacol **82**:253–256, 1993.
89. Acuna-Castroviejo D, Reiter RJ, Menendez-Pelaez A, Pablos MI, Burgos A. Characterization of high-affinity binding sites in purified cell nuclei of rat liver. J Pineal Res **16**:100–113, 1994.
90. Becker-Andre M, Wiesenberg I, Schaeren-Wiemers N, Andre E, Missbach M, Souret JH, Carlberg C. Pineal hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem **269**:28531–28534, 1994.
91. Carlberg C, Wiesenberg I. The orphan receptor family RZR/ROR, melatonin and 5-lipoxygenase: An unexpected relationship. J Pineal Res **18**:171–178, 1995.
92. Hardeland R, Reiter RJ, Poeggeler B, Tan DX. The significance of the metabolism of the neurohormone melatonin: Protection and formation of bioactive substances. Neurosci Biobehav Rev **17**:347–357, 1993.
93. Poeggeler B, Reiter RJ, Hardeland R, Sewerynek E, Melchiorri D, Barlow-Walden LR. Melatonin, a mediator of electron transfer and repair reactions, acts synergistically with the chain-breaking antioxidants ascorbate, trolox, and glutathione. Neuroendocrinol Lett **17**:87–92, 1995.
94. Huerto-Delgadillo L, Anton-Tay F, Benitz-King G. Effects of melatonin on microtubule assembly depend on hormone concentration: Role of melatonin as a calmodulin antagonist. J Pineal Res **13**:55–62, 1992.
95. Reiter RJ. Melatonin: That ubiquitously acting pineal hormone. News Physiol Sci **6**:223–227, 1991.
96. Vaughan GM, Taylor TJ, Pruitt BAJ, Mason ADJ. Pineal function in burns: Melatonin is not a marker for general sympathetic activity. J Pineal Res **2**:1–12, 1985.
97. Khoory R, Stemme D. Plasma melatonin levels in patients suffering from colorectal carcinoma. J Pineal Res **5**:251–258, 1988.
98. West SK, Oosthuizen JM. Melatonin levels are decreased in rheumatoid arthritis. J Basic Clin Physiol Pharmacol **3**:33–40, 1992.
99. Kennedy SH, Kutcher SP, Ralewski E, Brown GM. Nocturnal melatonin and 24-hour 6-sulphatoxymelatonin levels in various phases of bipolar effective disorder. Psychiatry Res **63**:219–222, 1996.
100. Pang SF, Allen AE. Extra-pineal melatonin in the retina: Its regulation and physiological function. In: Reiter RJ, Ed. Pineal Research Review. New York: Alan R. Liss, Vol **4**:pp55–95, 1986.
101. Huether G, Poeggeler B, Reimer A, George A. Effect of tryptophan administration on circulating melatonin levels in chicks and rats: Evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci **51**:945–953, 1992.
102. Itoh MT, Ishizuka B, Kudo Y, Fusama S, Amemiya A, Sumi Y. Detection of melatonin and serotonin N-acetyltransferase and hydroxyindole-O-methyltransferase in rat ovary. Mol Cell Endocrinol **136**:7–13, 1997.
103. Tijimes M, Pedraza R, Valladares L. Melatonin in the rat testis: Evidence for local synthesis. Steroids **61**:65–68, 1996.
104. Tan DX, Manchester LC, Reiter RJ, Qi W, Zhang M, Weintraub ST, Cabrera J, Sainz RM, Mayo JC. Identification of highly elevated levels of melatonin in bone marrow: Its origin and significance. Biochim Biophys Acta **1472**:206–214, 1999.
105. Tan D-X, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: A potent, endogenous hydroxyl radical scavenger. Endocr J **1**:57–60, 1993.
106. Poeggeler B, Reiter RJ, Hardeland R, Tan DX, Barlow-Walden LR. Melatonin and structurally related endogenous indoles act as potent electron donors and radical scavengers *in vitro*. Redox Rep **2**:179–184, 1996.
107. Matuszak Z, Reszka KJ, Chignell CF. Reaction of melatonin and related indoles with hydroxyl radicals: EPR and spin trapping investigations. Free Radic Biol Med **23**:367–372, 1997.
108. Susa N, Ueno S, Furukawa Y, Ueda J, Sugiyama M. Potent protective effect of melatonin on chromium (VI) -induced DNA single-strand breaks, cytotoxicity, and lipid peroxidation in primary cultures of rat hepatocytes. Toxicol Appl Pharmacol **144**:377–384, 1997.
109. Li XJ, Zhang LM, Gu J, Zhang AZ, Sun FY. Melatonin decreases production of hydroxyl radical during cerebral ischemia-reperfusion. Chung Kuo Yao Li Hsueh Pao **18**:394–396, 1997.
110. Tan DX, Manchester LC, Reiter RJ, Plummer BF, Hardies LJ, Weintraub ST, Vijayalaxmi Shepherd AMM. A novel melatonin metabolite, cyclic 3-hydroxymelatonin: A biomarker of *in vivo* hydroxyl radical generation. Biochem Biophys Res Commun **253**:614–620, 1998.
111. Stasica P, Ulanski P, Rosiak JM. Melatonin as a hydroxyl radical scavenger. J Pineal Res **25**:65–66, 1998.
112. Pähkla R, Zilmer M, Kullisaar T, Rägo L. Comparison of the antioxidant activity of melatonin and pinoline *in vitro*. J Pineal Res **24**:96–101, 1998.
113. Okada S, Nakamura N, Sasaki K. Radioprotection of intracellular genetic material. In: Nygaard OF, Simic MG, Eds. Radioprotectors and Anticarcinogens. New York: Academic Press, pp339–356, 1983.
114. Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F. Melatonin: A peroxyl radical scavenger more effective than vitamin E. Life Sci **55**:PL271–PL276, 1994.
115. Pieri C, Moroni F, Marra M, Marcheselli F, Recchioni R. Melatonin is an efficient antioxidant. Arch Gerontol Geriatr **20**:159–165, 1995.
116. Scaiano JC. Exploratory laser flash photolysis study of free radical reactions and magnetic field effects in melatonin chemistry. J Pineal Res **19**:189–195, 1995.
117. Marshall KA, Reiter RJ, Poeggeler B, Aruoma OI, Halliwell B. Evaluation of the antioxidant activity of melatonin *in vitro*. Free Radic Biol Med **21**:307–315, 1996.
118. Livrea MA, Tesoriere L, D'Arpa D, Morreale M. Reaction of melatonin with lipoperoxyl radicals in phospholipid bilayers. Free Radic Biol Med **23**:706–711, 1997.

119. Reiter RJ. Antioxidant actions of melatonin. Adv Pharmacol **38**:103–117, 1997.
120. Reiter RJ, Tan DX, Cabrera J, D'Arpa D, Sainz RM, Mayo JC, Ramos S. The oxidant/antioxidant network: Role of melatonin. Biol Signals Recept **8**:56–63, 1999.
121. Cagnoli CM, Atabay C, Kharlamov E, Manev H. Melatonin protects neurons from singlet oxygen-induced apoptosis. J Pineal Res **18**:222–226, 1995.
122. Gilad E, Cuzzocrea S, Zingarelli B, Salzman AL, Szabo C. Melatonin is a scavenger of peroxynitrite. Life Sci **60**:PL169–PL174, 1997.
123. Cuzzocrea S, Zingarelli B, Gilad E, Hake P, Salzman AL, Szabo C. Protective effect of melatonin in carrageenan-induced models of local inflammation: Relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. J Pineal Res **23**:106–116, 1997.
124. Noda Y, Mori A, Liburdy R, Packer L. Melatonin and its precursors scavenge nitric oxide. J Pineal Res **27**:159–163, 1999.
125. Poeggeler B, Saarela S, Reiter RJ, Tan DX, Chen LD, Manchester LC, Barlow-Walden LR. Melatonin—a highly potent endogenous radical scavenger and electron donor: New aspects of the oxidation chemistry of this indole assessed *in vitro*. Ann N Y Acad Sci **738**:419–420, 1994.
126. Mahal HS, Sharma HS, Mukherjee T. Antioxidant properties of melatonin: A pulse radiolysis study. Free Radic Biol Med **26**:557–565, 1999.
127. Reiter RJ. Oxidative processes and antioxidative defense mechanisms in the aging. FASEB J **9**:526–533, 1995.
128. Reiter RJ. Oxidative damage in the central nervous system: Protection by melatonin. Prog Neurobiol **56**:359–384, 1998.
129. Antolin I, Rodriguez C, Sainz RM, Mayo JC, Uria H, Kotler ML, Rodriguez-Colunga MJ, Tolivia D, Menendez-Pelaez A. Neurohormone melatonin prevents cell damage: Effect on gene expression for antioxidant enzymes. FASEB J **10**:882–890, 1996.
130. Kotler M, Rodriguez C, Sainz RM, Antolin I, Menendez-Pelaez A. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res **24**:83–89, 1998.
131. Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD, Poeggeler B. Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int **26**:497–502, 1995.
132. Pablos MI, Chuang JI, Reiter RJ, Ortiz GG, Daniels WMU, Sewerynek E, Melchiorri D, Poeggeler B. Time course of melatonin-induced increase in glutathione peroxidase activity in chick tissues. Biol Signals **4**:325–330, 1995.
133. Pablos MI, Guerrero JM, Ortiz GG, Agapito MT, Reiter RJ. Both melatonin and a putative nuclear melatonin receptor agonist CGP 52608 stimulate glutathione peroxidase and glutathione reductase activities in mouse brain *in vivo*. Neuroendocrinol Lett **18**:49–58, 1997.
134. Pablos MI, Reiter RJ, Ortiz GG, Agapito MT, Chuang JI, Sewerynek E. Rhythms of glutathione peroxidase and glutathione reductase in the brain of chick and their inhibition by light. Neurochem Int **32**:69–75, 1998.
135. Sewerynek E, Reiter RJ, Melchiorri D, Ortiz GG, Lewinski A. Oxidative damage in the liver induced by ischemia-reperfusion: Protection by melatonin. Hepatogastroenterology **43**:898–903, 1996.
136. Pierrefiche G, Laborit H. Oxygen radicals, melatonin, and aging. Exp Gerontol **30**:213–227, 1995.
137. Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, Honma K, Kondo T. Melatonin induces γ-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. Free Radic Biol Med **27**:838–847, 1999.
138. Montilla P, Tunez I, Munoz MC, Soria JV, Lopez A. Antioxidative effect of melatonin in rat brain oxidative stress induced by adriamycin. Rev Esp Fisiol **53**:301–305, 1997.
139. Bettahi I, Pozo D, Osuna C, Reiter RJ, Acuna-Castroviejo D, Guerrero JM. Physiological concentrations of melatonin inhibit nitric oxide synthase activity in the rat hypothalamus. J Pineal Res **20**:205–210, 1996.
140. Guerrero JM, Reiter RJ, Ortiz GG, Pablos MI, Sewerynek E, Chuang JI. Melatonin prevents increases in neural nitric oxide and cycle GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (*Meriones unguiculatus*). J Pineal Res **23**:24–31, 1997.
141. Pozo D, Reiter RJ, Calvo JM, Guerrero JM. Inhibition of cerebellar nitric oxide synthase and cyclic GMP production by melatonin *via* complex formation with calmodulin. J Cell Biochem **65**:430–442, 1997.
142. Waldhauser F, Dietzel M. Daily and annual rhythms in human melatonin secretion: Role in puberty control. Ann N Y Acad Sci **453**:205–214, 1985.
143. Tan D-X, Reiter RJ, Chen LD, Poeggeler B, Manchester LC, Barlow-Walden LR. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis **15**:215–218, 1994.
144. Manev H, Uz T, Kharlamov A, Joo JJ. Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. FASEB J **10**:1546–1551, 1996.
145. Antolin I, Obst B, Burkhardt S, Hardeland R. Antioxidative protection in a high-melatonin organism: The dinoflagellate *Gonyaulax polyedra* is rescued from lethal oxidative stress by strongly elevated, but physiologically possible concentrations of melatonin. J Pineal Res **23**:182–190, 1997.
146. Reiter RJ, Tan D-x, Kim SJ, Manchester LC, Qi W, Garcia JJ, Cabrera JC, El-Sokkary G, Rouvier-Garay V. Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. Mech Aging Dev **110**:157–173, 1999.
147. Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, Dink DT. Pineal melatonin rhythm: Reduction in aging Syrian hamsters. Science **210**:1372–1373, 1980.
148. Reiter RJ, Craft CM, Johnson JE Jr., King TS, Richardson BA, Vaughan GM, Vaughan MK. Age-associated reduction in nocturnal melatonin levels in female rats. Endocrinology **109**:1295–1297, 1981.
149. Reiter RJ. Pineal melatonin: Cell Biology of its synthesis and of its physiological interactions. Endocr Rev **12**:151–180, 1991.
150. Benot S, Molinero P, Soutto M, Goberna R, Guerrero JM. Circadian variations in the rat serum total antioxidant status: Correlation with melatonin levels. J Pineal Res **25**:1–4, 1998.
151. Benot S, Goberna R, Reiter RJ, Garcia-Maurino S, Osuna C, Guerrero JM. Physiological levels of melatonin contribute to the antioxidant capacity of human serum. J Pineal Res **27**:59–64, 1999.
152. Rodriguez AB, Marchena JM, Nogales G, Duran J, Barriga C. Correlation between the circadian rhythm of melatonin, phagocytosis, and superoxide anion levels in ring dove heterophils. J Pineal Res **26**:35–42, 1999.
153. Sainz RM, Mayo JC, Uria H, Kotler M, Antolin I, Rodriguez C, Menendez-Pelaez A. The pineal neurohormone melatonin prevents *in vivo* and *in vitro* apoptosis in thymocytes. J Pineal Res **19**:178–188, 1995.
154. Maestroni GJM, Covacci V, Conti A. Hematopoietic rescue *via* T-cell dependent, endogenous granulocyte-macrophage colony-stimulating factor induced by the pineal neurohormone melatonin in tumor-bearing mice. Cancer Res **54**:2429–2432, 1994.
155. Ruifrok ACC, Weil MM, Thames HD, Mason KA. Diurnal variations in the expression of radiation-induced apoptosis. Radiat Res **149**:360–365, 1998.
156. Tan D-X, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, Barlow-Walden LR. The pineal hormone melatonin inhibits DNA-adducts formation induced by the chemical carcinogen safrole *in vivo*. Cancer Lett **70**:65–71, 1993.
157. Reiter RJ, Tang L, Garcia JJ, Munoz-Hoyos A. Pharmacological actions of melatonin in free radical pathophysiology. Life Sci **60**:2255–2271, 1997.
158. Tang L, Reiter RJ, Li ZR, Ortiz GG, Yu BP, Garcia JJ. Melatonin reduces the increase in 8-hydroxydeoxyguanosine levels in the brain and liver of kainic acid treated rats. Mol Cell Biochem **178**:299–303, 1998.
159. Morioka N, Okatani Y, Wakatsuki A. Melatonin protects against age-related DNA damage in the brains of female senescence-accelerated mice. J Pineal Res **27**:202–209, 1999.
160. Qi W, Reiter RJ, Tan DX, Manchester LC, Kim SJ, Garcia JJ. Inhibitory effects of melatonin on ferric nitriloriacetate-induced lipid peroxidation and oxidative DNA damage in the rat kidney. Toxicology **139**:81–91, 1999.
161. Qi W, Reiter RJ, Tan DX, Garcia JJ, Manchester LC, Karbownik M, Calvo JR. Chromium (III)-induced 8-hydroxydeoxyguanosine in DNA and its reduction by antioxidants: Comparative effects of melatonin, ascorbate, and vitamin E. Environ Health Perspect **108**:399–402, 2000.

162. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuna-Castroviejo D. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res **18**:1–11, 1995.
163. Princ FG, Maxit AG, Cardalda C, Battle A, Juknat AA. *In vivo* protection by melatonin against δ-aminolevulinic acid–induced oxidative damage and its antioxidant effect on the activity of haem enzymes. J Pineal Res **24**:1–8, 1998.
164. Siu AW, Reiter RJ, To CH. Pineal indolamines and vitamin E reduce nitric oxide–induced lipid peroxidation in rat retinal homogenates. J Pineal Res **27**:122–128, 1999.
165. Tesoriere L, D'Arpa D, Conti S, Giacone V, Pintandi AM, Livrea MA. Melatonin protects human red blood cells from oxidative hemolysis: New insight into the radical-scavenging activity. J Pineal Res **27**:95–105, 1999.
166. Musatov SA, Anisimov VN, Andre V, Vigreux C, Godard T, Gauduchon P, Sichel F. Modulatory effects of melatonin on genotoxic response of reference mutagens in the Ames test and the comet assay. Mutat Res **417**:75–84, 1998.
167. Blickenstaff RT, Brandstadter SM, Reddy S, Witt R. Potential radioprotective agents: 1. Homologs of melatonin. J Pharm Sci **83**:216–218, 1994.
168. Vijayalaxmi Meltz ML, Reiter RJ, Herman TS, Kumar SK. Melatonin and protection from whole-body irradiation: Survival studies in mice. Mutat Res **425**:21–27, 1999.
169. Kaya H, Delibas N, Serteser M, Ulukaya E, Ozkaya O. The effect of melatonin on lipid peroxidation during radiotherapy in female rats. Strahlenther Onkol **175**:285–288, 1999.
170. Ahlers I, Solar P, Ahlersova E, Kassayova M, Smajda B. The influence of melatonin on metabolic changes in female rats induced by continuous irradiation and/or administration of 7,12-dimethylbenz/a/anthracene. Neoplasma **44**:253–257, 1997.
171. Vijayalaxmi Meltz ML, Reiter RJ, Herman TS. Melatonin and protection from genetic damage in blood and bone marrow: Whole-body irradiation studies in mice. J Pineal Res **27**:221–225, 1999.
172. Badr FM, El Habit OHM, Harraz MM. Radioprotective effect of melatonin assessed by measuring chromosomal damage in mitotic and meiotic cells. Mutat Res **444**:367–372, 1999.
173. Mohan N, Sadeghi K, Reiter RJ, Meltz ML. The neurohormone melatonin inhibits cytokine, mitogen, and ionizing radiation–induced NF-κB. Biochem Mol Biol Int **37**:1063–1070, 1995.
174. Kundurovic Z, Scepovic M. Histoenzymological reactions of the thyroid gland in irradiated and previously melatonin-treated irradiated rats. Acta Med Iugosl **43**:337–347, 1989.
175. Kundurovic Z, Mornjakovic Z. Morphometric characteristics of thyroid cells in irradiation-stressed rats treated with pinealectomy and melatonin. Med Arh **46**:9–10, 1992.
176. Mornjakovic Z, Scepovic M, Kundurovic Z. Morphometric aspects of seminiferous tubules in rats treated with melatonin and whole body irradiation. Med Arh **45**:9–10, 1991.
177. Mornjakovic Z, Alicelebic S, Bilalovic N, Susko I. Morphometric characteristics of Leydig cells after total irradiation of rats treated with melatonin. Med Arh **52**:183–184, 1998.
178. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicol Sci **50**:271–279, 1999.
179. Barchas J, DaCosta F, Spector S. Acute pharmacology of melatonin. Nature (London) **214**:919–921, 1967.
180. Ishizuka B, Kuribayashi Y, Murai K, Amemiya A, Itoh MT. The effect of melatonin on *in vitro* fertilization and embryo development in mice. J Pineal Res **28**:48-51, 2000.
181. Lieberman HR, Waldhauser F, Garfield G, Lynch HJ, Wurtman RJ. Effects of melatonin on mood and performance. Brain Res **323**:201–207, 1984.
182. Arendt M, Aldhous M, Marks V. Alleviation of jet lag by melatonin: Preliminary results of controlled double-blind trial. Br Med J Clin Res **292**:1170, 1986.
183. Lissoni P, Barni S, Crispino S, Tancini G, Fraschini F. Endocrine and immune effects of melatonin therapy in metastatic cancer patients. Eur J Cancer Clin Oncol **25**:789–795, 1989.
184. James SP, Sack DA, Rosenthal NE, Mendelson WB. Melatonin administration in insomnia. Neuropsychopharmacology **3**:19–23, 1990.
185. Sarnel A, Wegmann AM, Vejvoda M, Maass H, Gundel A, Schutz M. Influence of melatonin treatment on human circadian rhythmicity before and after a simulated 9-hour time shift. J Biol Rhythms **6**:235–248, 1991.
186. Voordouw BC, Euser R, Verdonk RE, Alberda BT, de Jong FH, Drogendijk AC, Fauser BC, Cohen M. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function and can inhibit ovulation. J Clin Endocrinol Metab **74**:108–117, 1992.
187. Lissoni P, Barni S, Rovelli F, Brivio F, Adrizzoia A, Tancini G, Conti A, Maestroni GJ. Neuroimmunotherapy of advanced solid neoplasm with single evening subcutaneous injection of low-dose interleukin-2 and melatonin: Preliminary results. Eur J Cancer **29**:185–189, 1993.
188. Nordlund JJ, Lerner AB. The effects of oral melatonin on skin color and on the release of pituitary hormones. J Clin Endocrinol Metab **45**:768–774, 1977.
189. Barsacchi R, Kusmic C, Damiani E, Carloni P, Greci L, Donato L. Vitamin E consumption induced by oxidative stress in red blood cells is enhanced by melatonin and reduced by N-acetyl-serotonin. Free Radic Biol Med **24**:1187–1192, 1998.
190. Marchiafava PL, Longoni B. Melatonin as an antioxidant in retinal photoreceptors. J Pineal Res **26**:184–189, 1999.