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Melatonin for Protection Against Ionizing Radiation

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1. Introduction

Radiations exist ubiquitously in the environment since the Earth's creation in soil, water and plants. Radiation exposure is a concern in the health industry and other occupations in the world. Apart from diagnostic, therapeutic and industrial purposes, humans also are exposed to ionizing radiations during air and space travel and exploration, background radiation, nuclear accidents, and nuclear terror attacks. Elevated radiation levels have been detected following Chernobyl on April 1986 at Ukraine, and recently Fukushima Daiichi Nuclear Power Plants on March 2011 at Japan. This raised the need for finding out efficient and reliable radioprotectors especially when a whole nation is exposed at high or even low levels for a prolonged period. The fallout and radioactivity cause concern during the weeks and months after the accidents. In addition, radiations are commonly used in a number of medical and industrial situations; however, their pro-oxidative effects limit their applications. Therefore, it is essential to protect humans from ionizing radiations by efficient pharmacological intervention. A valid approach to halt normal tissue radiotoxicity is the use of radioprotectors that when present prior to radiation exposure protect normal tissues from radiation effects. This view has also been used as a successful preventative measure for possible nuclear/radiological situation. From a practical point of view radioprotectors should perfectly have several criteria that relate to the ability of the agent to improve the therapeutic outcome. Ionizing radiation causes oxidative damage to tissues within an extremely short period, and possible protection against it would require the rapid transfer of smart antioxidants to the sensitive sites in cells. At this point, melatonin (N-acetyl-5-methoxytryptamine; MW= 232), an innate antioxidant produced mainly by the pineal gland, seems unique among antioxidants because of its multiple properties and reactions which reviewed and documented in several publications and summarised herein.

While ionizing radiation exposures, due to free radical generation, present an enormous challenge for biological and medical safety, melatonin is a potent radioprrpotector. In several investigations, melatonin has been recognized for successful amelioration of oxidative injury and illness due to direct and indirect effects of ionizing radiation and against oxidative stress in several experimental and clinical settings. Furthermore, numerous studies have

established that melatonin is a highly efficient free radical scavenger, broad antioxidant and stimulator of several antioxidants in biological systems. Because of its unique characteristics; melatonin has effects not only at the cell level but also within subcellular organelles and structures. The antioxidant and prophylatic properties of melatonin allow the use of radiation during radiotherapy to get better therapeutic outcomes. Several published articles documented that melatonin's anticancer and oncostatic effects make melatonin an excellent candidate and good choice to be used in routine radiotherapies, space travel and following nuclear accidents occupational settings where accidental exposure may occur. This article will review antioxidant features that put melatonin on top of potentially efficient pharmacological radioprotectors.

2. Ionizing radiation, free radicals & oxidative stress

Ionizing radiations are types of particle radiation (such as neutron, alpha particles, beta particles and cosmic ray) or electromagnetic (such as ultraviolet, X-rays and gamma rays) with sufficient energy to ionize atoms or molecules by detaching electrons from their valence orbitals. The degree and nature of such ionization depends on the energy of the individual particles or on frequency of electromagnetic wave. It is well known that exposure to ionizing radiation at sufficiently high doses results in various types of adverse biological effects. The biological effect of radiation involves direct and indirect actions. Both actions produce molecular changes that mostly need enzymatic repair. Indirect effect involves the production of reactive free radicals which produce oxidative mutilation on the key molecules. The environmental sources of oxidative attack include, in particularly, specific exposures of the organism to ionizing radiations like X-, γ- or cosmic rays and α-particles from radon decay as well as UVA and UVB solar light. Ionizing radiations prevalent in space, involve a broad range of radiation types and energies from cosmic and unpredictable solar sources, representing a very diverse range of ionization qualities and biological effectiveness. Linear energy transfer (LET) is a measure of the energy transferred to tissue or cells as an ionizing particle travels through it. The LET of the potential radiations can cover several orders of magnitude from <1.0 keV μm^{-1} to > several 100 keV μm^{-1} (Blakely and Chang 2007) Low LET radiation causes damage through reactive oxygen species (ROS) production mainly by the radiolysis of water present in living system.

From a chemical point of view, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are oxygen and nitrogen containing molecules constitute the main category of free radicals which may be defined as any chemical moiety generated with an unpaired number of electrons in valancy orbital. ROS include oxygen-based free radicals, the superoxide anion (O_2•−), hydroxyl (•OH), alkoxyl (RO•), peroxyl (ROO•), and hydroperoxyl (HOO•). RNS include peroxynitrite (ONOO−), nitric oxide (NO•), and nitrogen dioxide (NO2•). ROS may be radical, such as, O_2•− and •OH, or non-radical, such as, hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). At high concentrations, free radicals can be harmful to living organisms. Some ROS damage biomolecules indirectly. For example, H_2O_2 and O_2•− initiate DNA and lipids damage by interaction with transition metal ion, in particular iron and copper, in the metal-catalysed Haber–Weiss reaction, producing •OH. It is the most electrophilic and reactive of the ROS, with a half-life of~10^{-9} s (Draganic and Draganic 1971). •OH can be produced by ultraviolet and ionizing radiations (Von Sonntag 1987). This radical is considered the most frequently damaging species. It has been estimated that the •OH is responsible for 60–70% of the tissue damage caused by ionizing radiations (Galano

et al. 2011). Moreover, •OH has great ability to react with almost any molecule in the vicinity of where it is generated (Reiter et al. 2010). Chemical nature and reactivity of free radicals in biological systems has been recently reviewed (Galano et al. 2011). Once formed ROS and RNS can produce a chain reaction. The transfer of the free radical to a biological molecule can be sufficiently damaging to cause bond breakage or inactivation of key functions. The organic ROO• can transfer the radical from molecule to molecule causing damage at each encounter. Thus, a cumulative effect can occur, greater than a single ionization or broken bond.

A variety of external events, in particular, exposure to ionizing or ultraviolet radiation, can lead to an increase in the generation of ROS in comparison with available antioxidants leading to oxidative stress. Oxidative stress is caused by the presence of excessive amount of ROS which the cell is unable to counterbalance. This implies that the steady state balance of pro-oxidant/anti-oxidant systems in intact cells is shifted to the former. When excessive oxidative events occur, the pro-oxidants outbalance the anti-oxidant systems. Moreover, oxidative stress may result by overwhelming of antioxidant and DNA repair mechanisms in the cell by ROS. In radiation sickness oxidative stress is a factor as either cause or effect. The result is oxidation of critical cellular macromolecules including DNA, RNA, proteins and lipids eventually leading to cell death in severe oxidative stress. On the other hand, moderate oxidative stress may lead to activation of cytoplasmic/nuclear signal transduction pathways, modulation of gene and protein expression and alteration of DNA polymerase activity, affect the endogenous anti-oxidant systems by down-regulating proteins that participate in these systems, and by depleting cellular reserves of anti-oxidants (Acharya et al. 2010, Cadet et al. 2010, Little 2000).

3. Molecular biology effects of ionizing radiation due to free radical generation

As a matter of fact, ionizing radiation penetrating living tissue and can damage all important cellular components both through direct ionization and through generating ROS due to water radiolysis and induce oxidative damage. Radiation-induced oxidative stress was evaluated by three independent approaches; DNA damage, lipid peroxidation and protein oxidation.

3.1 DNA damage

Cells and their genomic constituent of the living organisms are continually exposed to oxidative attacks. Acute exposure to ionizing radiation can create oxidative stress in a cell and chronic exposure to this stress can result in permanent changes in the genome (Cooke et al. 2003). The main target of ionizing radiation has long since been indicated to be DNA which shows wide range of lesions. The oxidatively DNA damage commonly are apurinic/apyrimidinic (abasic) DNA sites, oxidized purines and pyrimidines, single strand (SSBs) and double strand (DSB) DNA breaks and non-DSB (Kryston et al. 2011). Other initial chemical events induced in DNA by ionizing radiation include cross-links, oxidative base modification (Hutchinson 1985) and clustered base damage (Goodhead 1994), sugar moiety modifications, and deaminated and adducted bases (Cooke et al. 2003, Sedelnikova et al. 2010, Sutherland et al. 2000, Ward 1994). The numbers of DNA lesions per cell that are detected immediately after a radiation dose of 1 Gy have been estimated to be approximately greater than 1000 base damage, 1000 SSBs, 40 DSBs, 20 DNA–DNA cross-

links, 150 DNA–protein cross-links and 160–320 non-DSB clustered DNA damage and defective DNA mismatch repair proteins (MMP) (Martin et al. 2010). Recently, it is suggested that radiation dose and the type of DNA damage induced may dictate the involvement of the MMP system in the cellular response to ionizing radiation. In particular, the literature supports a role for the MMP system in DNA damage recognition, cell cycle arrest, DNA repair and apoptosis (Martin et al. 2010). In addition, The DNA oxidation products are a direct risk to genome stability, and of particular importance are oxidative clustered DNA lesions, defined as two or more oxidative lesions present within 10 bp of each other (Sedelnikova et al. 2010).

The most common cellular DNA base modifications are 8-oxo-7,8-dihydroguanine (8-oxoGua) and 2,6- diamino-4-hydroxy-5-formamidopyrimidine. Both originate from the addition of the •OH to the C8 position of the guanine ring producing a 8-hydroxy-7,8-dihydroguanyl radical which can be either oxidized to 8-oxoGua or reduced to give the ring-opened FapyGua (Altieri et al. 2008, Kryston et al. 2011). The •OH interact with pyrimidines (thymine and cytosine) at positions 5 or 6 of the ring, and yield several base lesions. The most abundant and well known products, 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) and 5,6-dihydroxy-5,6-dihydrocytosine (cytosine glycol). It is generally accepted that 8-oxodG and thymine glycol are reliable biomarkers of high levels of oxidative stress and damage in the human body. These lesions ultimately are not lethal to the cell, but are considered to be highly mutagenic. Oxidized bases in DNA are potentially mutagenic and so are implicated in the process of carcinogenesis. It has been reported that X-radiation induced a significant increase in 8-OHdG concentration in mammary gland DNA (Haegele et al. 1998). High levels of 8-OHdG have been observed in normal human epidermis or purified DNA exposed to ultraviolet radiation (Wei et al. 1997). Thus, genome stability is crucial for maintaining cellular and individual homeostasis, but it is subject to many changes due to free radicals attack induced by the exposure to ionizing radiation. DNA breaks and fragments resulted from chromosomal damage appear as micronuclei in rapidly proliferating cells micronuclei frequency was markedly enhanced in bone marrow cells of mouse exposed to 5Gy radiation (Verma et al. 2010).

Comprehensive reviews have been appeared to give structural and mechanistic information on the radiation-induced damage to DNA (Cadet et al. 2010, Cadet et al. 2005). However, these authors showed that there is still a dearth of precise data on the formation of radiation-induced base injury to DNA in cells and tissues. This is because the determination of the radiation-induced base damage within DNA is achieved indirectly by methods utilizing hydrolysis of the biopolymer then followed by analysis of the free fragments. The situation is even more difficult for cellular DNA since highly sensitive assays are required to monitor the formation of very low amounts of injury, typically within the range of one modified base per 10^6 normal nucleotides.

Direct damage to DNA caused by ionizing radiation has been considered as a significant initiator of mutation and cancer. However, some reports suggest that extracellular and extranuclear targets may contribute to the genotoxic effects of radiation (Little 2000, Morgan 2003). In addition, it has been shown that irradiation of the cytoplasm produces gene mutations in the nucleus of the hit cells and that this process is mediated by free radicals (Wu et al. 1999). Recently, it is proposed that a possible extracellular signal-related kinase pathway involving ROS/RNS and COX-2 in the cytoplasmic irradiation-induced genotoxicity effect (Hong et al. 2010). Furthermore, it has been demonstrated that nitric oxide synthase (NOS) produces sustained high concentrations of nitric oxide (NO) in

various mammalian cells after exposure to radiation (Matsumoto et al. 2001). In cytoplasmic-irradiated cells, 3-nitrotyrosine, a nitrosated protein product used as a marker of ONOO-, was significantly elevated and dramatically inhibited by L-NMMA, (NO inhibitor) implicating a critical role of RNS in the mutagenicity induced by cytoplasmic irradiation (Hong et al. 2010).

3.2 Lipid peroxidation

The direct and indirect destructive effects of ionizing radiation lead to peroxidation of macromolecules, especially those present in lipid-rich membrane structures, lipoproteins and chromatin lipids. Phospholipids in membranes and triglycerides in LDL are highly susceptible to free radical attacks. Once the process of lipid peroxidation is started, it proceeds as a free radical-mediated chain reaction involving initiation, propagation, and termination (Gago-Dominguez et al. 2005). The first step (initiation) in the lipid peroxidation process is the abstraction of a hydrogen atom, from a methylene group next to a double bond in polyunsaturated fatty acids. This produces a carbon centered radical which undergo rearrangement of the double bond to form a stable conjugated diene. In propagation step, carbon centered radicals react with oxygen to form new ROO• that react further with another neighboring lipid molecule forming a hydroperoxy group and a new carbon centered radicals. The lipid hydroperoxide will react further to form cyclic peroxide, cyclic endoperoxide, and finally aldehydes. The propagation phase can repeat many times until it is terminated by chain breaking antioxidants (Halliwell 2009, Reed 2011).

During lipid peroxidative pathway, several end products are formed such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), pentane and ethane, 2,3 transconjugated dienes, isoprostanes and cholesteroloxides (Catala 2009, Tuma 2002). These aldehydes are highly reactive and bind with DNA and proteins and form adducts which inhibit proteins functions and can disrupt nuclear events. The aldehydes are more diffusible than free radicals, thus injury can occur in distant locations. The aldehyde can be found in measurable concentrations in biological fluids and analytical methods used are sometimes complex and require sample preparation involving extraction and purification steps. Isoprostanes, prostaglandin like compounds, are generated from the free radical-initiated peroxidation of arachidonic acid. F2-isoprostanes are the most specific markers of lipid peroxidation and the most difficult to measure (Comporti et al. 2008). The mostly frequently lipid peroxidation markers used in free radical research are MDA and 4-HNE. Lipid peroxidation was assessed as thiobarbituric acid reactive substances (TBARS) in biological materials using thiobarbituric acid reaction method (Esterbauer and Zollner 1989, Moller and Loft 2010).

Several studies have examined the radiation-induced free radical damage evidenced by the elevation of lipid peroxidation levels. Lipid peroxidation-derived products have been implicated in the pathogenesis of oxidative stress-associated radiation sickness and diseases. Aldehydes showed significant increase with increasing doses of ionizing radiation in several organs (Bhatia and Manda 2004, Sener et al. 2003) and mitochondrial membranes (Kamat et al. 2000). A significant increase in DNA strand breaks and TBARS concentrations was found in rat brain exposed to 10Gy ionizing radiation (Undeger et al. 2004). Exposure to 5 Gy irradiation led to considerable elevation of MDA level in thymus, brain, jejunum liver and kidney of total body irradiated mice (Taysi et al. 2003, Verma et al. 2010) after 24 hrs of irradiation continued up to 48 hrs. Lipid peroxidation due to •OH attack were found to be in a radiation dose-dependent manner but no significant differences between radiation

resistant and radiation sensitive rats were detectable after whole-body-irradiation with x-rays at 2, 4, and 6 Gy. Among the subcellular organelles mitochondria are one of the key components of the cell injured by radiation-induced oxidative stress. In an interesting study, mitochondria from rat brain and liver was isolated then exposed to 450Gy gamma radiation. In this study there was considerable increase in lipid hydroperoxide (LOOH) and MDA in rat liver and brain mitochondria (Lakshmi et al. 2005). Recent research showed that increased free radicals due to radiation exposure damage membrane lipids, which results in cell lysis due to altered membrane fluidity (Gulbahar et al. 2009).

The ideas about the leading role of lipid peroxidation in radiation damage of cells and tissues arose from the damaging of cell membrane structures. Peroxidation of lipids can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. It causes the change in structure, fluidity and permeability of membranes and inactivates several membrane associated enzymes and protein receptors. In biological membranes, lipid peroxidation is also usually accompanied by oxidation of membrane proteins. In consequence, peroxidation of lipids may change the agreement of proteins in bilayers and by that interfere with their physiological role on the membrane function.

3.3 Protein oxidation

As defined earlier, ionizing radiation can interact and modify all cellular components both through direct ionization and through induction of ROS resulting in a variety of subtle and profound biological effects. Radiation-induced oxidative protein damage can be started by even quite low doses of radiation and can produce an alteration of the cellular redox balance, which lasts for substantial time after exposure and may contribute to changes in cell survival, proliferation, and differentiation(Shuryak and Brenner 2009). Several damages to the peptide chain or to the side-chains of amino acid residues have been identified, and some of their mechanisms of formation has been described (Griffiths et al. 2002).

Available data from various studies raveled that the most sensitive amino acids, cysteine, tryptophan, tyrosine and methionine, bear aromatic or sulphur-containing side-chains. Furthermore, protein oxidation can lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and to conversion of some amino acid residues to carbonyl derivatives (Catala 2007). The fundamental mechanisms involved in the oxidation of proteins by ROS were described by studies in which amino acids, peptides, and proteins were exposed to ionizing radiations under conditions where •OH or a mixture of •OH and O_2•− are formed (Stadtman 2004). It has been demonstrated that the attack by •OH leads to an abstraction of a hydrogen atom from the protein polypeptide backbone and form a carbon-centered radical (Klaunig et al. 2011, Stadtman 2004). Oxidation due to radiation exposure can lead also to cleavage of the polypeptide chain and formation of cross-linked protein aggregates. Because the generation of carbonyl derivatives occurs by many different mechanisms, the level of carbonyl groups in proteins is widely used as a marker of oxidative protein damage (Guajardo et al. 2006). Studies performed with various tissues have revealed that radiation increases protein oxidation and carbonyl levels as well as produces structural and functional changes (Gulbahar et al. 2009).

In most reports describing the in vivo experiments on radiation sickness, the carbonyl levels were determined in tissue homogenates or soluble cytoplasmic proteins. Moreover, it is very

important to consider the carbonyl levels in different subcellular fractions, since they may show different susceptibility to oxidative damage, probably due to differences in their protein composition or activities of their antioxidant defenses, and, therefore, make different contribution to the impairment of cell functioning with radiation responses.
Numerous investigations showed that protein carbonyls, a marker of primary protein damage indicated a higher magnitude of damage in irradiated mice brains exposed to 1.5 Gy high-LET 56Fe beams (500 MeV/nucleon, 1.5 Gy). This effect was associated with impaired cognitive behavior of mice at day 30 post-exposure as well as apoptotic and necrotic cell death of granule cells and Purkinje cells (Manda et al. 2008). γ-irradiation of rats at a dose of 10 Gy caused increases in protein carbonyl groups in mitochondria and cytoplasm both in liver and spleen. Similar results have been obtained for homogenates of different tissues isolated from γ-irradiated gerbils and rats (Sohal et al. 1995). Post-irradiation accumulation of oxidized proteins in subcellular fractions, especially if occurring in nuclei, might probably affect not only the catalytic properties of enzymes but also the regulation of radiation-induced gene expression by interfering with the activation of transcription factors (Whisler et al. 1997). Among the nuclear proteins, histones are likely most susceptible to oxidative modification, due to high contents of lysine and arginine residues in their molecules. Information on the formation of radicals on peptides and proteins and how radical damage may be propagated and transferred within protein structures have been reviewed (Hawkins and Davies 2001).

4. Defenses against free radicals

Human and all of the aerobic organisms have a very efficient defense network of antioxidants against oxidative stress. An antioxidant can be defined as a molecule or an element that, when present at low concentrations compared to those of the oxidizable substrate, significantly combat, delays and inhibit oxidation of that substrate, thus, prevent free radicals from damaging healthy cells (Halliwell 1997, 2009). Under normal condition, cells have well coordinated and efficient endogenous antioxidant defense systems, which protect against the injurious effects of oxidants.
From the viewpoint of mechanistic functions, antioxidant defense mechanisms can be classified into the following five lines of defenses: preventing antioxidants, scavenging antioxidants, repair and de novo antioxidants, adaptive antioxidants, and finally cellular signaling messenger (Halliwell 1997, Niki 2010). The first line of defense is the preventing antioxidants which act by suppressing the formation of ROS and RNS by reducing H_2O_2 and lipid hydroperoxides that are generated during lipid peroxidation, to water and lipid hydroxides, respectively, or sequestering pro-oxidant metal ions such as iron and copper by some binding of proteins (e.g., transferrin, metallothionein). The second line of defense can be described as the scavenging antioxidants which exist to intercept, or scavenge free radicals and remove active species rapidly before attacking biologically essential molecules. For example; superoxide dismutase (SOD) converts $O_2\bullet-$ to H_2O_2, while α-tocopherol and carotenoids are efficient scavenger of $^1O_2$ (Inoue et al. 2011). Many phenolic compounds and aromatic amines act as a free radical-scavenging antioxidant. There is a general agreement that electron transfer and hydrogen transfer are the main mechanisms involved in the reactions of melatonin with free radicals. The third line of defenses is various enzymes which function by repairing damages, clearing the wastes, and reconstituting the lost function. The adaptation mechanism is considered the fourth line of defense, in which

appropriate antioxidants are released at the right time and transported to the right site in right concentration. Some antioxidants constitute the fifth line of defense by functioning as a cellular signaling messenger to control the level of antioxidant compounds and enzymes (Niki and Noguchi 2000, Noguchi and Niki 2000).

5. Radioprotectors and mitigators of radiation induced injury

Generally, any chemical/biological agents given before to or at the time of irradiation to prevent or ameliorate damage to normal tissues are termed radioprotectors. While mitigators of normal tissue injury are agents delivered at the end of irradiation, or after irradiation is complete, but prior to the manifestation of normal tissue toxicity. The estimated time scale to use mitigators efficiently ranges from seconds to hours after radiation exposure. Agents delivered to improve established normal tissue toxicity are considered treatments which can be monitored over weeks to years after radiation exposure (Citrin et al. 2010). Since radiotherapy, occupational, accidental exposure to radiation or space travel and exploration can produce unwanted side effects, it is important to prevent such effects by the use of radioprotectors or mitigators. Ideally, radioprotective and mitigative agent should fulfill several characteristics that relate to the ability of the agent to improve the therapeutic results. First, the agent should have protective effects on the majority of organs and tissues. Second, the agent must reach all cells and organelles and can easily penetrate cellular membranes. Third, it must have an acceptable route of administration (preferably oral or alternatively intramuscular) and with minimal toxicity. Fourth, to be useful in the radiotherapy settings, radioprotectant should be selective in protecting normal tissues from radiotherapy without protecting tumor tissue. Finally, to a large extent radioprotectors should be compatible with the wide range of other drugs that will be prescribe to patients. Moreover, because free radicals are responsible for injury caused by ionizing radiation, therefore, for an agent to protect cells from primary free radical damage, the agent needs to be present at the time of radiation and in sufficient concentration to compete with radicals produced through radical-scavenging mechanisms (Citrin et al. 2010, Hosseinimehr 2007, Shirazi et al. 2007).

A large body of literature describes radioprotection or mitigation with a variety of agents after total body or localized exposures. A complete and comprehensive review of these agents is outside the scope of this chapter. Herein, we briefly highlight melatonin that have been described as radiation protectors and mitigators, and attempt to focus on it with demonstrated or anticipated usefulness for therapeutic radiation exposures. As defined above, an ideal radioprotectors need to have radical-scavenging properties and can also exert broad antioxidant activity. Whereas all antioxidants cannot afford full radioprotection, melatonin verify most of the criteria needed for efficient radioprotector , mitigators and treatment agent with antioxidant potential, radical scavenging characteristics and stimulator of intrinsic antioxidants.

6. Melatonin

6.1 Synthesis, distribution, and metabolism

Melatonin synthesis in the pineal gland has been reviewed in significant detail (Reiter 2003). In summary, pinealocytes take up L-tryprophane from blood. Via several enzymatic steps including tryptophan 5-hydroxylation, decarboxylation, N-acetylation and O-methylation,

in that sequence, N-acetyl-5-methoxytryptamine (melatonin) is synthesized. It is secreted upon biosynthesis into the extracellular fluid to the general circulation from which it easily crosses various cellular membranes. It is secreted by the pineal gland and its levels have diurnal variation and also fluctuate with sleep stages. They are higher during night (Luboshitzky et al. 1999). The diurnal/nocturnal levels of blood melatonin can range between 8 ± 2 pg/mL (light phase) and 81 ± 11 pg/mL (dark phase). The synthesis and presence of melatonin have also been demonstrated in non-pineal tissues such as retina, Harderian gland, gastrointestinal track, testes, and human lymphocytes. Furthermore, the distribution of melatonin in the human being is very broad (Reiter 2003). Once synthesized, the majority of melatonin diffuses directly towards the cerebrospinal fluid of the brain's third ventricle, while another fraction is released into the blood stream where it is distributed to all tissues and body fluids (Cheung et al. 2006). It is found in serum, saliva (Cutando et al. 2011, Novakova et al. 2011), cerebrospinal fluids (Rousseau et al. 1999), and aqueous humor of the eye (Chiquet et al. 2006), ovarian follicular fluid, hepato-gastrointestinal tissues (Messner et al. 2001). Melatonin in the milk of lactating mothers exhibits a marked daily rhythm, with high levels during the night and undetectable levels during the day (Illnerova et al. 1993, Sanchez-Barcelo et al. 2011). Moreover, melatonin production is not confined exclusively to the pineal gland, but other tissues including retina, Harderian glands, gut, ovary, testes, bone marrow and lens also produce it (Esposito and Cuzzocrea 2010).

Melatonin has two important functional groups which determine its specificity and amphiphilicity; the 5-methoxy group and the N-acetyl side chain. In liver melatonin is metabolized by P- 450 hepatic enzymes, which hydroxylate this hormone at the 6- carbon position to yield 6- hydroxymelatonin which conjugated with sulfuric or glucuronic acid, to produce the principal urinary metabolite, 6-sulfatoxymelatonin. In the final stage, conjugated melatonin and minute quantities of unmetabolized melatonin are excreted through the kidney. In addition to hepatic metabolism, oxidative pyrrole-ring cleavage appears to be the major metabolic pathway in other tissues, including the central nervous system (Esposito and Cuzzocrea 2010).

A plethora of evidence suggests that melatonin mediates a variety of physiological responses through membrane and nuclear binding sites. In mammals, the mechanisms of action of melatonin include the involvement of high affinity G protein-coupled membrane receptors (MT1, MT2), cytosolic binding sites (MT3 and calmodulin), and nuclear receptors of the RZR/ROR family. Melatonin also has receptor-independent activity and can directly scavenge free radicals. A disulfide bond between Cys 113 and Cys 190 is essential for high-affinity melatonin binding to MT2 and possibly to MT1 receptors (Dubocovich and Markowska 2005). RZR/ROR family is expressed in a variety of organs. It presumably regulates the immune system and circadian cycles via the nuclear receptor and these also may be involved in its regulation of antioxidative enzymes (Cutando et al. 2011).

6.2 Melatonin and factors that determine antioxidant capacity

A number of criteria that characterize an ideal free radical scavenging antioxidant can be identified. First, because free radicals are highly reactive with very short half life time, therefore, an efficient antioxidant should be ubiquitous and present in adequate amounts in tissues and cells. Furthermore, the biological systems are heterogeneous in nature, which affects the action and efficacy of antioxidants. Both hydrophilic and lipophilic antioxidants act at respective site. Some antioxidants are present in free form, but others as metabolite or

in bound form (Niki 2010). In contrast to other antioxidants that are either hydrophilic or lipophilic, melatonin is an amphiphilic small size molecule (Giacomo and Antonio 2007). These features of melatonin allow it to cross all morphophysiological barriers and to interact with toxic molecules throughout the cell and its organelles, thereby reducing oxidative damage to molecules in both the lipid and aqueous environments of cells. Numerous articles documented that melatonin is widely distributed and found in all body fluids, organs, cells and organelles. Recently, melatonin levels in the cell membrane, cytosol, nucleus, and mitochondrion was found to vary over a 24-hr cycle, although these variations do not exhibit circadian rhythms. The cell membrane has the highest concentration of melatonin followed by mitochondria, nucleus, and cytosol (Venegas et al. 2011).

Second, an efficient antioxidant should react with most of free radicals because as it is well known that free radicals are variable in their biological, chemical and physical properties that involved in the oxidative stress. In functional terms, have reported that melatonin exerts a host of antioxidant effects that can be described as a broad spectrum antioxidant (Karbownik and Reiter 2000). Initially, (Hardeland et al. 1993a, Hardeland et al. 1993b, Tan et al. 1993) are the first who documented that melatonin is a remarkably potent scavenger of the particularly reactive, destructive, mutagenic and carcinogenic •OH. It is documented that, melatonin is a more efficient •OH scavenger than either glutathione or mannitol, and that melatonin reacts at a diffusion-controlled rate with the •OH. Thus, melatonin is probably an important endogenously produced antioxidant. Extensive studies have established that melatonin is much more specific than its structural analogs in undergoing reactions which lead to the termination of the radical reaction chain and in avoiding prooxidant, carbon or oxygen centered intermediates (Hardeland et al. 2011, Poeggeler et al. 2002, Tan et al. 1993). Besides the •OH, melatonin in cell-free systems has been shown to directly scavenge H_2O_2, $^1O_2$ and HOCl with little ability to scavenge the O_2•–. Furthermore, melatonin scavenges nitric oxide (NO•) and suppresses the activity of its rate limiting enzyme, nitric oxide synthase (NOS), thereby inhibiting the formation of the ONOO-. In addition, melatonin scavenges a number of RNS including ONOO- and peroxynitrous acid (ONOOH). Moreover, melatonin has proven to scavenge alkoxyl, peroxyl radicals. The peroxyl radical (POO•), which is formed during the complex process of lipid peroxidation, is highly destructive to cells, because, once formed, it can propagate the process of lipid peroxidation. Therefore, agents that neutralize the POO• radical are generally known as chain breaking antioxidants, is important to maintaining the optimal function of not only cell membranes, but of cells themselves. It is estimated that each molecule of melatonin scavenged four POO• molecules (Pieri et al. 1994, Pieri et al. 1995), which would make it twice as effective as vitamin E, the principal well known chain-breaking lipid antioxidant and POO• scavenger. The most important products of melatonin's interaction with H_2O_2, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N-acetyl-5-methoxykynuramine(AMK), and 6-hydroxymelatonin, are also a highly efficient radical scavenger (Maharaj et al. 2003). The cascade of reactions where the secondary metabolites are also effective scavenges is believed to contribute to melatonin's high efficacy in reducing oxidative damage.

Very relevant to the development of this chapter is an alternate concept proposed to explain the protective effects of melatonin at the level of radical generation rather than detoxification of radicals already formed. It has been suggested that if melatonin is capable of decreasing the processes leading to enhanced radical formation, this might be achieved by low concentrations of the indole (Hardeland et al. 2011). Therefore, the main sources of

free radicals should be investigated with regard to their modulation by melatonin and their sensitivity to the stressor. In addition to free radicals generated by leukocytes, mitochondria should be mentioned as main sources. In support of this suggestion, melatonin may also reduce free radical generation in mitochondria by improving oxidative phosphorylation, thereby lowering electron leakage and increasing ATP generation (Acuna-Castroviejo et al. 2001).

In addition to its direct free radical scavenging actions, melatonin influences both functional integrity of other antioxidative enzymes and cellular mRNA levels for these enzymes. Many studies documented the influence of melatonin on the activity and expression of the antioxidants both under physiological and under conditions of elevated oxidative stress. As an indirect antioxidant, melatonin stimulates gene expression and activity of SOD, thereby inducing the rapid conversion of $O_2\bullet^-$ to H_2O_2. The removal of $O_2\bullet^-$ by SOD also leads to a reduced formation of the highly reactive and damaging ONOO-. Catalase (CAT) and glutathione peroxidase (GSH-Px), enzymes that metabolizes H_2O_2 to H_2O, have also been shown to be stimulated by melatonin (Karbownik and Reiter 2000). In fact, it was demonstrated by several investigator that melatonin stimulates the rate-limiting enzyme, γ-glutamylcysteine synthase thereby increasing the level of an important endogenous antioxidant, glutathione (GSH) (El-Missiry et al. 2007, Othman et al. 2008, Urata et al. 1999) which, besides being a radical scavenger, is used by GSH-Px as a substrate to metabolize H_2O_2. In this process, GSH is converted to oxidized glutathione (GSSG). To maintain high levels of GSH, melatonin promotes the activity of glutathione reductase, which converts GSSG back to GSH. The possible intracellular mechanisms and pathways by which melatonin regulates antioxidant enzymes were reviewed (Rodriguez et al. 2004).

An added value of melatonin is that its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) also has remarkable antioxidant properties and redox potential (Tan et al. 2001). It is formed when melatonin interacts with ROS, in particular, $^1O_2$ and H_2O_2. AFMK can be then enzymatically converted, by CAT, to N1-acetyl-5-methoxykynuramine (AMK). Cyclic 3-hydroxymelatonin (C3-OHM) is another product formed from melatonin by its interactions with free radicals, (Tan et al. 1998), which can be further metabolized by free radicals to AFMK (Tan et al. 2003). All these findings indicate that AFMK is a central metabolite of melatonin oxidation especially in nonhepatic tissues.

Interestingly, melatonin was shown to prevent the loss of important dietary antioxidants including Vitamins C and E (Susa et al. 1997), bind iron and participate in maintaining iron pool at appropriate level resulting in control of iron haemostasis, thereby providing tissue protection (Othman et al. 2008). Furthermore, melatonin enhances antioxidant action of a-tocopherol and ascorbate against NADPH- and iron-dependent lipid peroxidation in human placental mitochondria (Milczarek et al. 2010).

The bioavailability is the main factor that determines the capacity of antioxidants in vivo. The antioxidants should be absorbed, transported, distributed, and retained properly in the biological fluids, cells and tissues (Cheeseman and Slater 1993, Niki 2010). Recently, it has been suggested that melatonin present in edible plants may improve human health, by virtue of its biological activities and its good bioavailability (Iriti et al. 2010). This could add a new factor to the of health benefits for patients associated to radiotherapy. Melatonin's interactions with other drugs that influence its bioavailability were summarized (Tan et al. 2007). Furthermore, melatonin can be administered by virtually any route, including orally, via submucosal or transdermal patches, sublingually, intranasally, intravenously (Reiter 2003).

6.3 Radioprotective effect of melatonin and its metabolites

The radiosensitivity of cells to ionizing radiation depends on several factors including the efficiency of the endogenous antioxidative defense systems to prevent oxidative stress. A number of natural and synthetic compounds have been investigated for their antioxidative as well as radioprotective potential. Most of the effective compounds were found to have some inconvenient side effects such as hypotention, hypocalcemia, nausea, vomiting and hot flashes. Furthermore, most of compounds must be given intravenously or subcutaneously which restrict their clinical application outside of controlled clinical situations. Thus, a need still exists for identifying a non-toxic, effective, and convenient compound to protect humans against radiation damage in accidental, occupational, clinical settings and space-travel. Melatonin received much attention for its unique antioxidative potential at a very low concentration compared with other antioxidants.

A number of in vitro and in vivo studies have reported that exogenously administered melatonin provides profound protection against radiation induced lipid peroxidation and oxidative stress (El-Missiry et al. 2007, Taysi et al. 2003). As indicated above, radiation-induced lipid peroxidation is a three steps free radical process. Melatonin inhibits lipid peroxidation by preventing the initiation phase of lipid peroxidation and interrupting the chain reaction. This is mainly due to melatonin ability to quench •OH and several other ROS and RNS. It has been reported to scavenge the several ROS, in particular •OH and the ONOO-•. When melatonin interacts with •OH it becomes indolyl (melatonyl) radical which has very low toxicity. After some molecular rearrangements, the melatonyl radical scavenges a second •OH to form cyclic 3-hydroxymelatonin (3-OHM). Thus, this reaction pathway suggests that 3-OHM is the footprint product of the interaction between melatonin with •OH. 3-OHM was also detected in the urine of both rats and humans. This provides direct evidence that melatonin, under physiological conditions, functions as an antioxidant to detoxify the most reactive and cytotoxic endogenous •OH (Tan et al., 1999). When rats were exposed to ionizing radiation which results in •OH generation, urinary 3-OHM increased significantly compared to that of controls (Tan et al. 1998). This provided direct evidence that radiation induced oxidative stress increases melatonin consumption in rats. The rapid decrease in circulating melatonin under conditions of excessive stress can be considered a protective mechanism for organisms against highly damaging free radicals; in this sense, melatonin can be categorized as a first line of defensive molecule (Tan et al. 2007). Along this line, the melatonin's metabolite AFMK protected against space radiation induced impairment of memory and hippocampal neurogenesis in adult C57BL mice (Manda et al. 2008). This study demonstrated that radiation exposure (2.0 Gy of 500 MeV/nucleon ^{56}Fe beams, a ground-based model of space radiation) significantly reduced the spatial memory of mice without affecting their motor activity. It is also reported that AFMK pretreatment significantly ameliorated radiation induced neurobehavioral ailments and reduced the loss of doublecortin and cell proliferation. Radiation exposure dramatically augmented the level of 8-OHdG in serum as well as DNA migration in the comet tail were impaired by AFMK pretreatment. In addition, radiation-induced augmentation of protein carbonyl content and 4-HNE + MDA and reduced the level of brain sulfhydryl contents was ameliorated by AFMK pretreatment (Manda et al. 2008). The ameliorating action of AFMK against radiation induced lipid peroxidation was attributed to free-radical scavenging property of AFMK. In vitro, AFMK showed a very high level of •OH scavenging potential which was measured by an electron spin resonance spin study of the 2-hydroxy-5,5-dimethyl-1-pyrrolineN-oxide (DMPO-OH) adduct. In this experiment, 10 Gy of X-ray for the radiolysis of water with

different concentration of AFMK was used and intensity of spin adduct (DMPO-OH) were measured by ESR (Manda et al. 2007).

Extensive studies have established that pretreatment with melatonin at physiological dose 5 mg/kg or pharmacological dose 10 mg/kg bw significantly decreased MDA and NO• levels. The data documented that melatonin reduces tissue damage inflicted by irradiation when given prior to the exposure to ionizing radiation (Babicova et al. 2011, Taysi et al. 2003, Verma et al. 2010). These authors explained that NO• is formed in higher amounts from L-arginine by inducible nitric oxide synthase (iNOS) during early response to ionizing radiation presumably as a part of signal transduction pathways (Babicova et al. 2011). Its cytotoxicity is primarily due to the production of ONOO-, a toxic oxidant, generated when the NO• couples with O_2•- (El-Sokkary et al. 2002). The processes triggered by ONOO- include initiation of lipid peroxidation, inhibition of mitochondrial respiration, inhibition of membrane pumps, depletion of GSH, and damage to DNA. Melatonin is reported to scavenge ONOO- both in vitro and in vivo (El-Sokkary et al. 1999, Gilad et al. 1997) and to inhibit iNOS activity thereby reducing excessive NO• generation.

It should emphasized that ionizing radiation causes oxidative damage to tissues within an extremely short period, and possible protection against it would require the rapid transfer of antioxidants to the sensitive sites in cells. At this point, melatonin seems unique among cellular antioxidants because of its physical and chemical properties; it can easily cross biological membranes and reach the cytosol, nucleus, and cellular compartments (Menendez-Pelaez and Reiter 1993). The effect of melatonin in maintaining normal hepatic and renal functions may be related to its ability to localize mainly in a superficial position in the lipid bilayer near the polar heads of membrane phospholipids (El-Sokkary et al. 2002).

The protective action of melatonin against lipid and protein oxidation as a factor modifying membrane organization may also be related to melatonin's ability to scavenge the oxidation-initiating agents, which are produced during the oxidation of proteins and lipids. Since membrane functions and structure are influenced by proteins in membranes and radiation is known to damage thiol proteins (Biaglow et al. 2003), it is possible that the protective action of melatonin against membrane damage may be related partially to the ability of melatonin to prevent lipid and protein oxidative damage (Le Maire et al. 1990). Changes in membrane structure and fluidity due to ROS reactivity after irradiation are also attributed to graded alterations in the lipid bilayer environment (Karbownik and Reiter 2000). It has been suggested that the protective role of melatonin in preserving optimal levels of fluidity of the biological membranes may be related to its ability to reduce lipid peroxidation (Garcia et al. 1997, Garcia et al. 2001).

Moreover, melatonin prevents inflammation and MDA caused by abdominopelvic and total body irradiation of rat (Taysi et al. 2003). Thus, the radioprotective effect of melatonin is likely achieved by its ability to function as a scavenger for free radicals generated by ionizing radiation. Furthermore, these findings may suggest that melatonin may enable the use of higher doses of radiation during therapy and may therefore allow higher dose rates in some patients with cancer. In another study, pretreatment with melatonin (10mg/kg bw) for 4 days before acute γ-irradiation significantly abolished radiation induced elevations in MDA and protein carbonyl levels in the liver and significantly prevented the decrease in hepatic GSH content, GST, and CAT activities. Moreover, preirradiation treatment with melatonin showed significantly higher hepatic DNA and RNA contents than irradiated rats. The levels of total lipids, cholesterol, triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), total proteins, albumin, total globulins, creatinine, and urea,

as well as the activities of AST, ALT, and GGT in serum were significantly ameliorated when melatonin was injected before irradiation. The protection evidenced by normalization of the clinical parameters was associated with and attributed to decreased lipid peroxidation in the presence of melatonin. This data indicate that melatonin has a radioprotective impact against ionizing-radiation induced oxidative stress and organ injury (El-Missiry et al. 2007).

At sufficiently high radiation doses, GSH becomes depleted, leaving highly reactive ROS, beyond the immediate and normal needs of the cell, to react with critical cellular biomolecules and cause tissue damage. The concentration of intracellular GSH, therefore, is the key determinant of the extent of radiation-induced tissue injury. Thus, interest has been focused on melatonin, which acts as an antioxidant and is capable of stimulating GSH synthesis. It has been shown that melatonin enhances intracellular GSH levels by stimulating the rate-limiting enzyme in its synthesis, γ-glutamylcysteine synthase, which inhibits the peroxidative enzymes NOS and lipoxygenase. Experimentally, melatonin is demonstrated to increase hepatic GSH content, (El-Missiry et al. 2007, Sener et al. 2003), and hence to inhibit formation of extracellular and intracellular ROS (Reiter et al. 2004). It is also proposed that prevention of GSH depletion is the most efficient method of direct protection against radiation-induced oxidative toxicity. A significant decrease in hepatic GST activity and increase in serum GGT activity was recorded after exposure to 2&4 Gy (El-Missiry et al. 2007, Sridharan and Shyamaladevi 2002), and 10 Gy gamma irradiation (Samanta et al. 2004). Melatonin treatment at a dose level of 10mg/Kg bw before irradiation significantly countered radiation-induced decrease in the activities of these enzymes in the liver and serum. Furthermore, melatonin increases the activity of GSH-Px (Barlow-Walden et al. 1995) and superoxide dismutase (Antolin et al. 1996). These findings support the conclusion that melatonin affords radioprotection by modulating antioxidative enzyme activities in the body (El-Missiry et al. 2007).

Al the level of clinical markers, like cholesterol, TG, LDL, and free radicals are also risk factors that tend to damage arteries, leading to cardiovascular diseases. Pre-irradiation treatment with melatonin is found to reduce serum cholesterol, TG, and LDL levels in serum, indicating modulation of lipid metabolism in cells. It is well reported that antioxidants reduce oxidation susceptibility of HDL (Schnell et al. 2001) and control hyperlipidaemia (Mary et al. 2002). This might potentiate antiatherogenic effects of antioxidants including melatonin. Therefore, it is suggested that preirradiation treatment with melatonin appears to be hypolipidemic which might potentate its beneficial use in occupational, clinical, and space settings (El-Missiry et al. 2007).

Ionizing irradiation is among reproductive harmful agent and is widely identified to affect testicular function, morphology and spermatogenesis. Irradiation of the testes can produce reversible or permanent sterility in males. In an experiment, rats were subjected to sublethal irradiation dose of 8 Gy, either to the total body or abdominopelvic region using a ^{60}Co source. In this experiment, melatonin pretreatment resulted in less apoptosis as indicated by a considerable decrease in caspace-3 immunoreactivity. Electron microscopic examination showed that all spermatogenic cells, especially primary spermatocytes, displayed considerably inhibited of degeneration in the groups treated with melatonin before total body and abdominopelvic irradiation (Take et al. 2009).

An extensive literature implicates cellular DNA as the primary target for the biological and lethal effects of ionizing radiation. Melatonin has the ability to protect the DNA of hematopoietic cells of mice from the damaging effects of acute whole-body irradiation (Vijayalaxmi et al. 1999). The radioprotective ability of melatonin was investigated in the

Indian tropical rodent, Funambulus pennanti during its reproductively inactive phase when peripheral melatonin is high and the animal is under the influence of environmental stresses. Exogenous melatonin with its anti-apoptotic and antioxidant properties additively increased the immunity of the squirrels, by protecting their hematopoietic system and lymphoid organs against X-ray radiation induced cellular toxicity (Sharma et al. 2008).

Human keratinocytes is the main target cells in epidermal photodamage. Protection against UVR-induced skin damage was manifested by suppression of UV-induced erythema by topical pretreatment with melatonin with / without combination of vitamins C and E (Bangha et al. 1997, Dreher et al. 1998). Melatonin increased cell survival of HaCaT keratinocytes and ensured keratinocyte colony growth under UV-induced stress and showed decrease of UV-induced DNA fragmentation. Also, transcription of several classical target genes which are up-regulated after UV-exposure and play an important role in the execution of skin photodamage were down-regulated in HaCaT keratinocytes by melatonin pretreatment. It has been previously reported that melatonin reduces UVB-induced cell damage and polyamine levels in human skin fibroblasts (Lee et al. 2003). Furthermore, it was reported that melatonin increases survival of HaCaT keratinocytes by suppressing UV-induced apoptosis (Fischer et al. 2006). The molecular mechanisms underlying protective effects of melatonin on human keratinocytes and human fibroblasts upon UVB induced-apoptotic cell death was investigated (Cho et al. 2007). In this study, cDNA microarray analysis was perform from HaCaT keratinocytes, exposed to 100 mJ /cm2 and pretreated with melatonin for 30 min. Data showed that melatonin inhibits the expression of apoptosis related protein-3, apoptotic chromatin condensation inducer in the nucleus, and glutathione peroxidase 1 in UVB-irradiated HaCaT cells. The inhibitory effect of melatonin upon UVB irradiation is likely to be associated with antioxidant capacity of melatonin, thereby suggesting that melatonin may be used as a sunscreen agent to reduce cell death of keratinocytes after excessive UVB irradiation.

Radiotherapy plays an important role as part of the multimodality treatment for a number of malignancies in children. In young children, significant growth arrest was demonstrated with fractionated doses of 15 Gy and above as well as, in children less than one year of age, with doses as low as 10 Gy (Robertson et al. 1991). Proliferating chondrocytes, distal metaphyseal vessels, and epiphyseal vasculature are main targets for radiation-induced injury of bone growth plate (Kember 1967). Proliferating chondrocytes show marked cytological changes and cell death with a single fraction of 5 Gy. Melatonin with its antioxidant capacity protected against the hypoxic conditions of chondrocytes and promoted continued proliferation despite exposure to radiation. Moreover, in vitro studies showed that melatonin is capable of promoting osteoblast proliferation directly and stimulating these cells to produce increased amounts of several bone matrix proteins such as bone sialoprotein, alkaline phosphatase, osteocalcin (Roth et al. 1999) and procollagen type I c-peptide (Nakade et al. 1999), responsible for bone formation. Osteoprotegerin, an osteoblastic protein that inhibits the differentiation of osteoclasts is also increased by melatonin in vitro (Koyama et al. 2002). This data may support the radioprotective effect of melatonin on bone growth. The effects of fractionated radiotherapy combined with radioprotection by melatonin compared with fractionated radiotherapy alone in preserving the integrity and function of the epiphyseal growth plate from radiation damage in a weanling rat model was investigated. Data revealed that melatonin is more protective for bone growth protection than amifostine and a potential exists to implement the use of melatonin in an effort to maximize the radiotherapeutic management of children with less long-term morbidity than previous clinical experience (Yavuz et al. 2003).

Recent studies have documented that radiation in general and radiotherapy in particular has effects on brain function, such as thinking, memory and learning ability (Hsiao et al. 2010). Because cognitive health of an organism is maintained by the ability of hippocampal precursors to proliferate and differentiate, radiation exposures have been shown to inhibit neurogenesis and are associated with the onset of cognitive impairments. In recent investigation, on the protection by melatonin against the delayed effects of cranial irradiation on hippocampal neurogenesis melatonin maintained adult hippocampal neurogenesis and cognitive functions after irradiation (Manda and Reiter 2010). In this study the pretreatment with melatonin showed a significantly higher count of microtubule binding protein doublecortin and the proliferation marker Ki-67 positive cells compared with irradiated only animals. The protection was achieved by a single intraperitoneal injection of 10 mg melatonin/kg bw prior to irradiation. These protective effects were accompanied by significant control of oxidative stress indicated by reduction in the count of immunohistochemical localization of DNA damage and lipid peroxidation using the anti-8-hydroxy-2-deoxyguanosin the anti-hydroxynonenal. This indicated that melatonin minimize cell death.

6.4 Melatonin modulates apoptosis in radiotherapy and space radiation

Recent studies have showed the exposure to heavy ions such as ^{56}Fe or ^{12}C particle can induce detrimental physiological and histological changes in the brain, which lead to behavioral changes, spatial learning, and memory deficits. During space travel, astronauts are exposed to high-LET galactic cosmic rays at higher radiation doses and dose rates than humans received on Earth is one of the acknowledged showstoppers for long duration manned interplanetary missions. Hadrontherapy is an innovative modality of high precision tool for radiotherapy which consists in using hadrons (mainly protons or carbon ions) to irradiate tumors. This technique is used in certain cases to treat patients whose tumors are resistant to conventional X-ray radiotherapy. Given cancer therapy and space radiation protection, there is a demand for reliable agent for the protection of the brain against oxidative stress induced by heavy-ion radiation. It is well known that ionizing radiations can induce apoptosis. It is well known that oxidative stress is a mediator of apoptosis by compromising the fine balance between intracellular oxidant and their defense systems to produce abnormally high levels of ROS. Melatonin supplementation at 1,3&10mg/Kg bw reduced irradiation-induced oxidative damage, and stimulated the activities of SOD & CAT together with total antioxidant capacity in brain of rats exposed to heavy-ion radiation. Furthermore, pretreatment with melatonin significantly elevated the expression of Nrf2 which regulates redox balance and stress. In addition, pre-irradiation treatment with melatonin mitigated apoptotic rate, maintained mitochondrial membrane potential, decreased cytochrome C release from mitochondria, down-regulated Bax/Bcl-2 ratio and caspase-3 levels, and consequently inhibited the important steps of irradiation-induced activation of mitochondrial pathway of apoptosis (Liu et al. 2011). Studies performed by other investigators documented that melatonin pretreatment inhibited the cerebellum cell apoptosis after mice received 2 Gy ^{56}Fe particle irradiation (Manda and Reiter 2010) and that decreased apoptosis by melatonin was associated with a reduction in Bax/Bcl-2 ratio in mice splenocytes exposed to 2 Gy X-ray irradiation (Jang et al. 2009). Along this line, melatonin supplementation suppresses NO-induced apoptosis via induction of Bcl-2 expression in immortalized pineal PGT-β cells (Yim et al. 2002). A similar pathway of inhibitory effects of melatonin on apoptosis induced by ischemic neuronal injury has been determined (Sun et al. 2002).

Relevant to this context, it has been recently proposed that ERK MAPK plays a central role to determine whether cells will live or die in response to apoptotic stimuli. It is well documented that the apoptotic signaling activated during UVB stress mainly converges at the mitochondrial level into intrinsic pathway and supporting evidence consider this pathway might be the principle target of melatonin to prevent apoptosis in human leukocytes (Radogna et al. 2008) as well as in other tumor cell lines and in vivo models (Acuna-Castroviejo et al. 2007). An in vitro stress model for the cell protection and antiapoptotic functions of melatonin was studied using U937 cells exposed to UVB radiation (Luchetti et al. 2006). Melatonin sustained the activation of the survival-promoting pathway ERK MAPK (extracellular signal-regulated kinase) which controls the balance between survival and death-promoting genes throughout the MAPK pathway, and is required to antagonize UVB induced apoptosis of U937 cells. This kinase was found to modulate the antioxidant and mitochondrial protection effects of melatonin that may find therapeutic applications in inflammatory and immune diseases associated with leukocyte oxidative stress and accelerated apoptosis (Luchetti et al. 2009, Luchetti et al. 2006). Recently, it is reported that redox-sensitive components are included in the cell protection signaling of melatonin and in the resulting transcriptional response that involves the control of NF-κB, AP-1, and Nrf2. Through these pathways, melatonin stimulates the expression of antioxidant and detoxification genes, acting in turn as a glutathione system promoter (Luchetti et al. 2010).

It is suggested that ionizing radiation produces oxidative stress due formation of mitochondrial ROS resulting in calcium influx into the mitochondria with opening of the mitochondrial permeability transition pore (MPTP) (Andrabi et al. 2004, Halestrap 2006) and depolarization of the mitochondrial membrane potential as the end result of radiation-induced mitochondrial damage and cell apoptosis. The different regulatory mechanisms of apoptosis and their modification by treatment with melatonin were tested in different cells after irradiation. It is found that the mitochondrial pathway was strongly influenced by melatonin by reducing mitochondrial ROS generation and calcium release as well as inhibiting the opening of the MPTP as shown in rat brain astrocytes (Jou et al. 2004), mouse striatal neurons (Andrabi et al. 2004) and rat cerebellar granule neurons (Han et al. 2006). Moreover, the prevented decreases in the mitochondrial membrane potential resulted from irradiation suggests that melatonin, due to its physiochmeical characters crosses the blood–brain barrier and biological membranes to easily reach mitochondria, stabilizes oxidative stress-mediated dysfunction and integrity of mitochondria by preserving its membrane potential and increasing the efficiency of mitochondrial electron transfer chain and ATP synthesis (Acuna-Castroviejo et al. 2001). When melatonin treated cultured keratinocytes were irradiated with UVB radiation (50 mJ/cm2), there were less cell leaky, more uniform shape and less nuclear condensation as compared to irradiated, nonmelatonin-treated controls (Fischer et al. 2006). Exogenous melatonin with its anti-apoptotic and antioxidant properties additively increased the immunity of the animals, by protecting their hematopoietic system and lymphoid organs against X-ray radiation induced cellular toxicity (Sharma et al. 2008). These findings strongly highlight melatonin as a potential antiaopoptotic neuroprotective and mitigative agent against the space radiation hazards and the side effect risk in hadrontherapy. Consistent with all the overwhelming experimental evidence described above it may be concluded that melatonin can efficiently protect against and mitigate radiation induced oxidative stress. The majority of the published works on its ROS scavenging action coincide on the conclusion that melatonin is excellent for this task and make melatonin efficient pharmacological radioprotectors.

7. Toxicity and biosafety of melatonin

The melatonin doses chosen in several studies were between 5 and 15 mg/kg bw, which are rather minimal effective doses as reported in animal studies. Whereas, pharmacological studies in rats of up to 250 mg/kg doses did not indicate any adverse effects. In addition, human volunteers who ingested a single oral dose of 1–300 mg and 1 g of melatonin daily for 30 days did not report any adverse side effects. In a study, none of the 15 weanling rats administered with 5–15 mg/kg of melatonin died during the 6-wk observation period (Yavuz et al. 2003). In addition, ip treatment with melatonin for 45 days did not show abnormal singes (El-Missiry et al. 2007). All of these observations provide support for the non-toxic nature of melatonin (Cheung et al. 2006).

8. Conclusion

Apart from nuclear accidents, radiation has been used increasingly in medicine and industry to help with diagnosis, treatment, and technology. However, radiation hazards present an enormous challenge for the biological and medical safety. The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation ROS in cells as a result of water radiolysis leading to oxidative stress. •OH considered the most damaging of all free radicals generated in organisms, are often responsible for biomolecular damage caused by ionizing radiation. Oxidative stress greatly contributes to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during occupational settings, radiotherapy, and space flight. Melatonin is an indoleamine hormone synthesized from tryptophan in pinealocytes. It is distributed ubiquitously in organisms and in all cellular compartments, and it easily passes through all biological membranes. Several studies have indicated that melatonin may act as a scavenger of ROS such as hydroxyl radical, alkoxyl radical, hypochlorous acid and singlet oxygen. A number of in vitro and in vivo studies have reported that exogenously administered melatonin provides protection against radiation induced oxidative stress in different species. Its ability to reduce DNA damage, lipid peroxidation, and protein damage may originate from its function as a preventive antioxidant (scavenging initiating radicals directly or indirectly). Furthermore, this indoleamine manifests its antioxidative properties by upregulation of endogenous antioxidant defense mechanisms, increases the efficiency of the electron transport chain thereby limiting electron leakage and free radical generation, protects the integrity of the mitochondria and promotes ATP synthesis. Furthermore, several metabolites that are formed when melatonin neutralizes damaging reactants are themselves scavengers suggesting scavenging cascade reaction that greatly increase the efficacy of melatonin in preventing oxidative damage. Several observations provide support for the non-toxic nature of melatonin. The radioprotective and mitigative effects of melatonin against cellular damage caused by oxidative stress and its low toxicity make this innate antioxidant a potential drug in situations where the effects of ionizing radiation are to be controlled.

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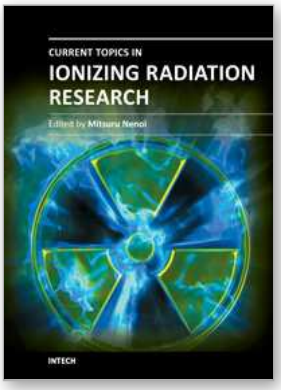

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Since the discovery of X rays by Roentgen in 1895, the ionizing radiation has been extensively utilized in a variety of medical and industrial applications. However people have shortly recognized its harmful aspects through inadvertent uses. Subsequently people experienced nuclear power plant accidents in Chernobyl and Fukushima, which taught us that the risk of ionizing radiation is closely and seriously involved in the modern society. In this circumstance, it becomes increasingly important that more scientists, engineers and students get familiar with ionizing radiation research regardless of the research field they are working. Based on this idea, the book "Current Topics in Ionizing Radiation Research" was designed to overview the recent achievements in ionizing radiation research including biological effects, medical uses and principles of radiation measurement.

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