Melatonin Role in Ameliorating Radiation-induced Skin Damage: From Theory to Practice (A Review of Literature)

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ABSTRACT

Normal skin is composed of epidermis and dermis. Skin is susceptible to radiation damage because it is a continuously renewing organ containing rapidly proliferating mature cells. Radiation burn is a damage to the skin or other biological tissues caused by exposure to radiofrequency energy or ionizing radiation. Acute skin reaction is the most frequently occurring side effect of radiation therapy. Generally, any chemical/biological agent given before or at the time of irradiation to prevent or ameliorate damage to normal tissues is called a radioprotector. Melatonin is a highly lipophilic substance that easily penetrates organic membranes and therefore is able to protect important intracellular structures including mitochondria and DNA against oxidative damage directly at the sites where such a kind of damage would occur. Melatonin leads to an increase in the molecular level of some important antioxidative enzymes such as superoxide, dismotase and glutation-peroxidase, and also a reduction in synthetic activity of nitric oxide. There is a large body of evidence which proves the efficacy of Melatonin in ameliorating UV and X ray-induced skin damage. We propose that, in the future, Melatonin would improve the therapeutic ratio in radiation oncology and ameliorate skin damage more effectively when administered in optimal and non-toxic doses.

Keywords

Radiation, Melatonin, Radiotherapy, Skin Damage

Radiation and Skin

Normal skin has two main sublayers including epidermis and the dermis [1]. It is estimated that half of the canceric patients undergo radiotherapy as a part of their treatment [2]. Skin is prone to radiation damage, because it is a continuously renewing organ containing rapidly proliferating mature cells [3].

Radiation burn is defined as a damage to the skin or other biological tissues caused by exposure to radiofrequency energy or ionizing radiation. High exposure to X-rays during repeated diagnostic medical imaging, interventional radiology procedures or radiotherapy can also cause radiation damage. The most common radiation burn is the therapeutic one [4-6]. Radiation burn properties include: First, radiation burns have a dose-dependent clinical pattern. Second, radiation burns are associated with opiate-resistant chronic pain. Third, unpredictable successive inflammatory waves which may occur weeks up to years after radiation

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exposure [3]. Despite all the care, unintentional radiation burns can occur with X-ray or RT machines as a result of mechanical problems, electrical instability, human errors, etc. We can exemplify Bailystok accident [4].

Acute skin reaction is the most common side effect of radiation therapy with statistics between 90-95% of patients experiencing some degrees of reaction [3,5]. Skin reactions are graded by severity n a continuum from erythema and dry desquamation to the moist desquamation and in more severe cases, ulceration in which threshold doses for the onset of each of these reactions are listed in Table 1 [1,6,7].

Radioprotectors in General

In general, any chemical/biological agent given prior to or at the time of irradiation to prohibit or improve damage to the normal tissues is called a radioprotector since radiotherapy, occupational and accidental exposure to radiation or space travel and exploration can induce unwilling side effects. It is important to prevent such effects by means of radioprotectors or mitigators.

Table 1: Threshold skin entrance doses and onset of various skin injuries [7].

Effect	Dose(Gy)	Onset
Early transient erythema	2	Hours
Main erythema	6	~10days
Temporary Epilation	3	~3Wk
Permanent Epilation	7	~3Wk
Dry desquamation	14	~4Wk
Moist desquamation	18	>4wk
Secondary Ulceration	24	>6wk
Late erythema	15	~8-10wk
Ischemic dermal necrosis	18	>10wk
Dermal atrophy(2nd Phase)	10	>12wk
Dermal atrophy(1st Phase)	10	>1yr
Induration (invasive fibrosis)	10	
Telangiectasia	10	>1yr
Late dermal necrosis	>12?	>1yr
Skin cancer	-	>5yr

Ideally, a radioprotector should benefit several properties including; first, the agent should have protective effects on lots of organs and tissues. Second, the agent should easily penetrate membranes into the cells environment. Third, it must have an accepted way of injection and be less toxic. Fourth, it should be applicable in radiotherapy treatment. Finally, to a large extent, radioprotectors should be compatible with lots of other drugs which are prescribed to patients in the process of their treatment [8,9,10].

After discovery of cysteine in 1949, a large number of chemical compounds with radiation protection capabilities have been studied. Some of these newly discovered drugs such as mercaptoethylamine (MEA), cystamine and WR2721 were considered as the most effective radioprotectors [11]. Due to some side effects such as: drowsiness, hypotension, Nausea, vomiting and toxicity, these drugs had limited clinical use, and attempts for finding new radioprotectors with low levels of toxicity are on the rise [12].

Melatonin and its General Properties

Melatonin was discovered in 1917 as an endogenous agent produced by the pineal gland. During past decades, scientists revealed that there were other organs which secrete Melatonin. These organs included bile fluid, bone marrow, cerebrospinal fluid, ovary, eye, lymphocytes, gastral mucosa and the skin. Melatonin is a highly lipophilic substance which easily penetrates organic membranes and is able to protect important intracellular structures including mitochondria and DNA against oxidative stress. Melatonin can cross all morpho-physiological obstacles including placenta and BBB. Its concentration in the body is typically low during daylight hours and high at night (Figure 1). There is clear evidence which sup-

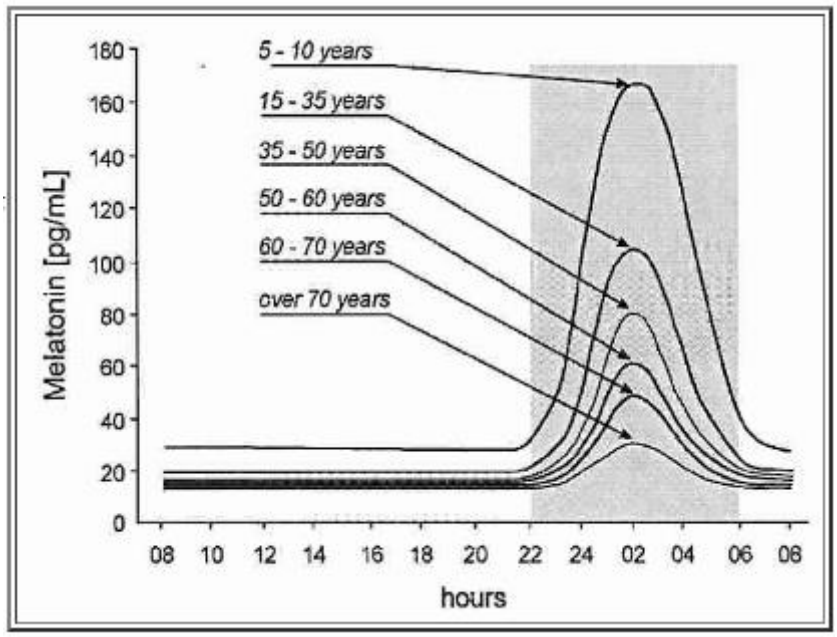

Figure 1: Melatonin concentration in proportion with age and time per pg/ml unit [31]

ports the claim that Melatonin level is conversely proportional to the age in mammals, including man. Moreover, seasonal variations contribute to the production of Melatonin in humans. The levels probably are higher in Winter as compared to Summer [12-15].

In addition, Melatonin contributes mainly to hair growth cycles, cutaneous pigmentation and skin physiology and pathology [14,16]. When examined in fur-producing animals, it was obvious that Melatonin could stimulate hair growth, considerably. A clinical trial in women suffering from androgenetic or diffuse alopecia cleared positive effect of Melatonin in human hair growth. The data suggeste that Melatonin can adjust hair growth in humans [16].

Melatonin Receptors

Melatonin receptors are categorized into two major groups including membrane-bond receptors and nuclear receptors. Membrane-bond receptors are divided into 3 subgroups including MT1, MT2 and MT3. In C57BL/6 mouse skin, MT2 is expressed uniquely, whereas, both receptors are expressed in human skin although with uneven proportion and a bias toward MT1. In skin cells, receptor-independent Melatonin actions might be interceded partly by cytosolic flavoprotein quinone reductase II (NQO2), which is specific to skin cells. NQO2 is associated with cellular resistance to oxidative stress and detoxification.

Nuclear receptor RORa (retinoid-related orphan receptor) is a member of the RZR/ROR subfamily which includes at least four splicing variants: RORa1, RORa2, RORa3 and RZRa (RORa4).

RORa1 is expressed only in adult dermal fibroblasts, whereas the type RORa2 is expressed in immortalized melanocytes and RORa4 (RZRa) is expressed in adult epidermal keratinocytes, HaCaT keratinocytes, neonatal melanocytes and adult dermal fibroblasts (Figure 2)[13-16].

Melatonin Optimal Dose

Melatonin can be injected through different

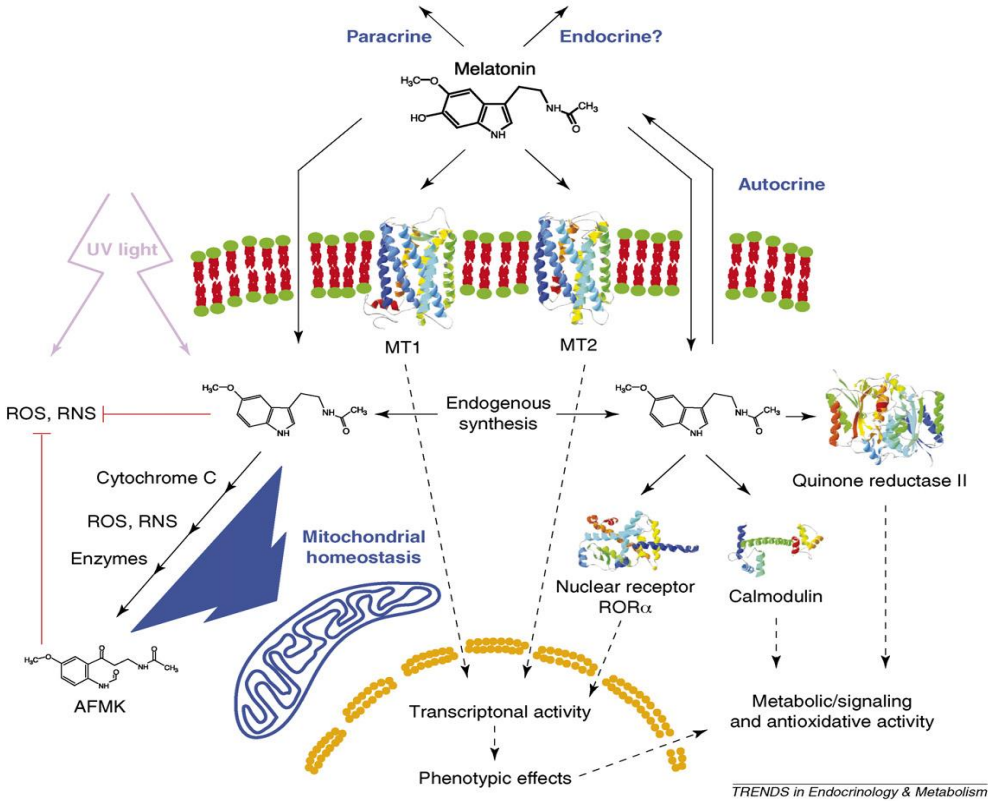

Figure 2: Pleiotropic effects of Melatonin in skin cells. Exogenous or endogenously synthesized Melatonin can regulate skin-cell phenotype through an interaction with membrane-bound Melatonin receptors MT1 and MT2 or with nuclear retinoid-acid orphan receptors (RORa). Among phenotypic effects of receptor activation, it is worth noting melanin-synthesis inhibition, DNA repair and overexpression of antioxidative enzymes. Non-receptor actions are mediated through an interaction with intracellular proteins, such as quinone reductase 2 (NQO2) or caldmodulin. Melatonin and its metabolites, represented in this figure by N1-acetyl-N2-formyl-methoxykynuramine (AFMK), act as direct scavengers of reactive oxygen and nitrogen species (ROS and RNS) and help to maintain mitochondrial homeostasis by interacting with cytochrome C or the electron-transport chain. In the skin, intra-, para- and auto-crine actions are envisioned, however, endocrine effects cannot be ruled out entirely. Direct effects are shown by solid lines and multiple reactions and signaling are shown by broken lines [15].

routes including oral, sub-mucosal, transdermal, sub-lingual, respiration and intravenously.

In order to determine Melatonin toxicity potential, physiologic to pharmacologic concentrations have been tested in different animals. Doses of Melatonin tested in vivo were as follows: 10–250 mg/kg in mice, 100–200 mg/kg in rats or even 800 mg/kg in mice, rabbits, cats and dogs. However, doses as low as 0.1 mg/kg administered via oral route were believed to be effective and appropriate minimum dose level of Melatonin. It has been reported that Melatonin (at a dose as high as 250 mg/kg) is not considered toxic anymore.

As an important note, Melatonin use is not illegal in the USA (i.e. it does not require US FDA confirmation) for treatment of androgen-

ic alopecia and as a component of sunscreens [14-17].

Melatonin as a Free Radical Scavenger and Antioxidant

Melatonin is produced almost in all organisms ranging from plants to humans. Melatonin shows free-radical scavenging and antioxidative properties in all species. Melatonin increases the molecular level of some significant antioxidative enzymes such as superoxide dismotase and glutation-peroxidase and also reduces synthetic activity of nitric oxide.

According to Tan et al. study, Melatonin is more effective than glotatione or manythole in the process of OH free radical scavenging (respectively 5, 14 fold). Melatonin is also a potent free-radical scavenger relative to vitamin C and throlox.

Melatonin is an important antioxidant and effective scavenger of hydroxyl radical (•OH). (Figure 3)[13-18].

Melatonin and Skin Damage

In 1998, Dreher et al. administered vitamin C, vitamin E and Melatonin alone or in combination, topically 30 min. prior to ultraviolet-irradiation of the skin. The results showed a mild protective effect of such vitamins when applied alone and a dose-dependent photoprotective effect of Melatonin. Better protection was reached by using the combination of Melatonin with both vitamins [19]. In 2000, Nickle et al. studied the effect of Melatonin on human keratinocytes which were exposed to UV radiation. Significant protection against UVB-induced decrease in DNA synthesis was reported as compared to the irradiated control group without Melatonin supplementation. This effect was directly proportional to the applied Melatonin concentration. Interestingly, no protective effect of Melatonin was observed after UVA irradiation [20]. In 2001, Kim et al. investigated the effects of X-ray irradiation and Melatonin on cytotoxicity, lipid peroxidation and alteration of the cell cycle in cultured skin fibroblast. By pre-incubation with Melatonin (10^{-5} M), a significant preventive effect was reported on the increase in the absolute number of surviving cells (up to 68% of cells survived), and the levels of MDA considerably reduced. DNA flow-cytometry analysis revealed that X radiation increased pre-G1 apoptotic population by 7.6% compared to a very low level (1.3%) of non-irradiated cells. However, by pretreatment with Melatonin, this apoptotic population decreased up to 4.5% at 10^{-5} M [21].

In 2001, Ryoo et al. studied the effect of Melatonin on UVB irradiated cultured dermal fibroblasts. By pre-cultivation with Melatonin (10^{-9} M), a significant preventive effect was reported on the increase in the absolute number of surviving cells (up to 92.5% of cells survived), and the levels of MDA considerably decreased. UVB limits G1 progression induced pre-G1 arrest leading to apoptotic changes of dermal fibroblast, and those are stopped by Melatonin pre-treatment [22]. In 2005, Hussein et al. studied radioprotective effects of Melatonin against X-ray-induced skin damages in albino rats ultrastructurally. As compared to non-irradiated skin, XRI skin demonstrated signs of cellular damage in the basal, spinous and granular cells. These features included destruction of epidermal cells, reduced irregularity of the basal cell borders, swollen mitochondria, dilated RER, decreased complements of cytoplasmic organelles and shortage of desmosomes. Additionally, irradiated cells exhibited early apoptotic changes such as reduction in the cytoplasmic and nuclear areas, extensive cytoplasmic vacuolization, abnormal ER, mitochondria and condensation of the nuclear chromatin. Results revealed that Melatonin could minimize all XRI-induced ultrastructural skin damage parameters [23]. In 2006, Fischer et al. studied the effect of Melatonin on the survival of HaCaT cells. DNA synthesis experiments showed a strong

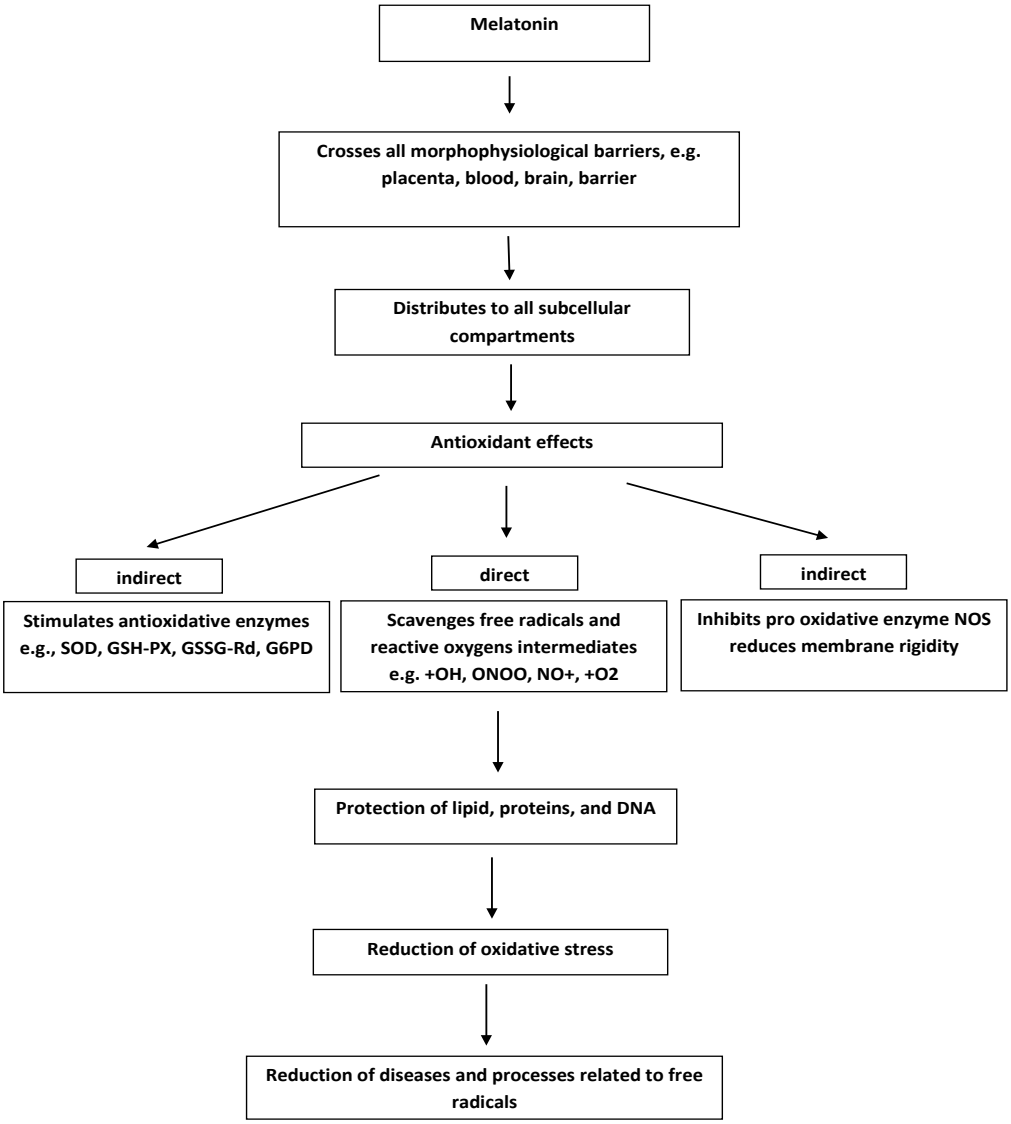

Figure 3: Presumed actions of Melatonin as a direct free radical scavenger and as an indirect antioxidant. In this review only the direct detoxification of reactive oxygen and nitrogen species by Melatonin are considered. However, Melatonin also has been shown to have indirect antioxidative actions, through the stimulation of several antioxidative enzymes and the stabilization of membrane fluidity. In both in vitro and in vivo studies, Melatonin reduced free radical damage to lipids, proteins, and DNA. Because of the role of oxidative damage in disease processes, antioxidants, including Melatonin may help to resist the development of various pathophysiologies [17].

protective effect by pre-treatment with Melatonin at concentrations of 10^{-4} M and 10^{-3} M. The mechanism of Melatonin protective effect (10^{-6} to 10^{-3} M) consists of inhibition of apoptosis as measured by TUNEL assay. Clonogenic studies demonstrated a significantly higher number of colonies in cultures treated with Melatonin compared to controls. Pre-treatment with Melatonin led to significant protection against UVB-induced damage in keratinocytes [24].

In 2009, Izykowska et al. studied the effect

of Melatonin after its addition to the culture medium for 30 minutes before exposure of melanoma cells to UVA or UVB. Melatonin added to the medium at concentrations of 10^{-3} to 10^{-8} M was found to increase the number of melanoma cells as compared to the control after 24 hours. At 10^{-3} M Melatonin, melanoma cells irradiated by 30 mJ/cm^2 UVB increased in number and at 10^{-9} M increased the survival of those exposed to 60 mJ/cm^2 UVB. The cells exposed to UVA (15 J/cm^2) were protected by Melatonin at 10^{-6} and 10^{-9} M[25]. In 2009, Izykowska et al. evaluated the effect of Melatonin added to culture medium 30 minutes before exposure of keratinocytes and fibroblasts to irradiation with UVA and UVB. Melatonin at 10^{-3} M increased the number of surviving keratinocytes and at 10^{-6} M increased the number of surviving fibroblasts irradiated by UVB as compared to cells exposed only to radiation. Melatonin at 10^{-3} M showed a protective effect on both types of cells irradiated with UVA [26]. In 2014, Cakir et al. studied radioprotective effects of Melatonin on the skin histology in rats. As a result, the histological abnormalities of radiation are shaped to be better protected in skin through pretreatment with Melatonin [27].

In 2014, Janjetovic et al. investigated the protective effects of Melatonin and its derivatives in human keratinocytes against a range of doses of ultraviolet B radiation. There was significant reduction in the production of reactive oxygen species (50–60%) when UVB-exposed keratinocytes were treated with Melatonin or its metabolites. Similarly, Melatonin and its derivatives decreased the nitrite and hydrogen peroxide levels induced by UVB as early as 30 min. after the exposure. In addition, Melatonin and its metabolites enhanced the levels of reduced glutathione in keratinocytes within 1 hr. after UVB exposure in comparison with control cells. They also observed a dose-dependent increase in viability of UVB-irradiated keratinocytes which were treated with Melatonin or its metabolites after 48 hr. MMelatonin and its derivatives enhanced the DNA repair capacity of UVB-induced pyrimidine photoproducts or cyclobutane pyrimidine dimers production in human keratinocytes. Melatonin and its metabolites further reinforced expression of p53 phosphorylated at Ser-15 but not at Ser-46 or its non-phosphorylated form [28]. In 2014, Ozguner et al. studied the effect of Melatonin on mobile-phone-induced skin tissue changes. In IR group, increased thickness of stratum corneum, atrophy of epidermis, papillamatosis, basal cell proliferation, increased granular cell layer (hypergranulosis) in epidermis and capillary proliferation, impairment in collagen tissue distribution and separation of collagen bundles in dermis were all reported compared to the control group. Most of these changes, except hypergranulosis, were prohibited by Melatonin administration [29]. Cho et al. performed cDNA microarray analysis from keratinocytes that were pre-incubated with Melatonin and then irradiated by 100 mJ/cm^2 UV irradiation. A great variety of genes related to apoptosis, cancer induction, cyclin-dependent kinase 2–interacting protein, GPx, ubiquitin-conjugating enzyme E2M enzymes and signal transducer genes (fibroblast growth factor, TGFβ-stimulated protein TSC 22) were underexpressed by Melatonin compared with UV-exposed keratinocytes [30].

Conclusion

Melatonin is a multifunctional hormone. This agent has different features such as direct and indirect scavenging of free radicals, the ability to stimulate the activity of antioxidant enzymes and inhibiting the activity of a pro-oxidative enzyme making it a potentially useful radioprotector. There are ample of in-vitro and in-vivo studies which demonstrate its protection against radiation injury.

Radiotherapy is a frequent and effective form of cancer therapy. Acute skin reaction is the most frequently occurring side effect of ra-

diation therapy. Melatonin might be successfully used for the prevention and treatment of radiation-induced skin injury.

Based on radiobiological models, we can assume that Melatonin might delay the saturation of repair enzymes which leads to repairing more radiation-induced damage by repair system and more importantly providing the possibility of administering higher doses of radiation during radiotherapy to get a better therapeutic ratio.

We propose that, in the future, Melatonin would improve the therapeutic ratio in radiation oncology and ameliorate skin damages more effectively when administered in optimal and non-toxic doses.

Conflict of Interest

None

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