

# Radio-protective role of antioxidant agents

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## Abstract

Ionizing radiation interacts with biological systems to produce reactive oxygen species and reactive nitrogen species which attack various cellular components. Radio-protectors act as prophylactic agents to shield healthy cells and tissues from the harmful effects of radiation. Past research on synthetic radio-protectors has brought little success, primarily due to the various toxicity-related problems. Results of experimental research show that antioxidant nutrients, such as vitamin E and herbal products and melatonin, are protective against the damaging effects of radiation, with less toxicity and side effects. Therefore, we propose that in the future, antioxidant radio-protective agents may improve the therapeutic index in radiation oncology treatments.

## Introduction

The ability of potent protective agents to provide protection against the damaging effects of ionizing radiation was first reported in 1949.<sup>1</sup> Research efforts with synthetic radio-protectors in the past brought lit-

tle success primarily due to the various toxicity-related problems.<sup>2</sup> The most efficient radio-protective agents that were originally tested against lethal doses of X rays and gamma radiation in mice is WR-2721, also known as amifostine.<sup>2</sup> Although amifostine was reported to be an effective radio-protector in clinical radiation oncology, it was later found to cause some undesirable side effects, such as hypotension, vomiting, nausea, sneezing, hot flashes, mild somnolence and hypocalcemia.<sup>3</sup>

Results obtained from animal experiments show that antioxidant nutrients, such as vitamin A, C and E,<sup>4</sup> and a large number of herbal productions<sup>5</sup> are protective against lethality and other radiation effects.<sup>4</sup> Furthermore, a large number of papers have been published confirming the ability of melatonin, a pineal gland hormone, to protect against radiation-induced damage.<sup>3,6,7</sup>

This review briefly covers the effects of irradiation on biological systems and presents various features that make antioxidants a potent radio-protector in radiation oncology trials.

## Radiobiological considerations

Exposure of biological systems to ionizing radiation leads to formation of reactive oxygen species (ROS) and reactive nitrogen species.<sup>8</sup> These reactive species damage the various bio-macromolecules, like the DNA, lipids and proteins present in the cell.<sup>9</sup> Furthermore, exposure to high doses of ionizing radiation results in damage to the hematopoietic, gastrointestinal or central nervous systems, depending on radiation dose (Table 1)<sup>6,10</sup> Since the hematopoietic system has a high level of cell turnover; it has among the most radiosensitive tissues in the body.<sup>11,12</sup> Gastrointestinal and central nervous syndromes are induced by a higher irradiation dose compared to hematopoietic syndrome.<sup>13</sup> An understanding of the biological effects of irradiation is necessary in order to understand the important role of radio-protective agents.

## Antioxidant nutrients

Antioxidant agents, such as vitamin A, C and E, offer protective properties against radiation because deleterious effects of radiation mimics the oxidative damage associated with active oxygen toxicity.<sup>14</sup> Selenium (as Na<sub>2</sub>SeO<sub>3</sub>) and vitamin E have been reported to act alone and in an additive form as radio-protective and chemo-preventive agents.<sup>15</sup>

Ebselen, a seleno-organic compound, has been shown to protect cells against the damaging effects of radiation caused by ROS.<sup>16</sup> When

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cells were exposed to 2 Gy and 20 Gy of gamma irradiation, there was a significant difference between the untreated cells and the cells pre-treated with ebselen with respect to apoptotic features and mitochondrial function. Ebselen administration for 14 days at a daily dosage of 10 mg/kg provided significant protection against killing and oxidative damage to mice exposed to whole-body gamma irradiation. These data show that ebselen may have great potential as a new class of *in vivo*, non-sulfur containing radio-protectors.<sup>16</sup>

Many animal studies have recently been published on the radio-protective effects of propolis.<sup>17</sup> Propolis is a resinous substance collected by honey bees. Ethanolic extract of propolis is effective in reducing and delaying radiation-induced mucositis in an animal model.<sup>17</sup>

Results from animal experiments indicate that antioxidant nutrients are protective against lethality and other radiation effects but to a lesser degree than most synthetic radio-protectors, such as amifostine. However, some antioxidant nutrients have the advantage of low toxicity even though they are generally protective when administered at pharmacological doses.<sup>4</sup>

## Vitamin E

Vitamin E (alpha tocopherol) and related analogs are nutraceuticals that can scavenge singlet oxygen and superoxide anion radicals.<sup>18</sup> Vitamin E has been shown to maintain jejunal, ileal, and colonic fluid absorption in irradiated rats,<sup>18</sup> even when treatment with vitamin E after 1 Gy radiation exhibited protective effects against radiation induced chromosomal aberrations and micronuclei in mouse bone marrow.<sup>19</sup> A water-soluble vitamin E derivative, tocopherol monoglucoside (TMG), was reported to be very effective in protecting DNA both *in vitro* and also in mice after oral or intraperitoneal administration against gamma irradiation.<sup>20</sup>

TMG [(2- $\alpha$ -D-glucopyranosyl) methyl-2, 5, 7, 8-tetramethylchroman-6-ol], was efficient when administered immediately after radiation exposure at a dose of 600 mg/kg. The treatment decreased radiation-induced abnormal metaphases in mouse bone marrow chromosomes and reduced the frequency of micronucleated erythrocytes at 24 h after exposure to radiation.<sup>21</sup> TMG administered before exposure to radiation also protected against radiation-induced teratogenesis in mice.<sup>22</sup>

Noaman *et al.*<sup>23</sup> showed that vitamin E and selenium (Se) protect antioxidant enzymes in irradiated animals. In this study, rats were injected intraperitoneally with 200 mg/kg body weight (body wt.) of vitamin E and sodium selenite (0.1 mgSe/kg body wt.) for ten days before exposure to gamma radiation at 6.5 Gy. Radiation-induced depressions in blood glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), and increases in plasma lipid peroxide products were normalized in the irradiated rats that were

treated with combined vitamin E and Se.<sup>23</sup>

The extent of changes in antioxidant enzymes levels after irradiation may differ according to animal tissue, but vitamin E and Se administration tends to normalize the enzyme levels in irradiated tissue.<sup>24</sup> Also, most studies support other evidence for the synergistic effects of vitamin E and Se in protecting against the oxidative damage caused by radiation.<sup>25</sup> However, further studies are needed to confirm these radio-protective effects.

## Herbal radio-protectors

The majority of synthetic radio-protectors have limited clinical application owing to their side effects and inherent toxicity.<sup>13,26,27</sup> Therefore, natural herbal radio-protectors, such as *Ginkgo biloba*, Chaga mushroom, green tea (polyphenols), dithiolthiones, *Panax ginseng*, Shigoka extract, and *Spirulina platensis*, have become an important alternative.<sup>27</sup>

The proposed radio-protective efficacy of plant products is due to their containing a large number of active constituents, such as antioxidants, immunostimulants and compounds with antimicrobial activity.<sup>27</sup> Citrus extract, at a dose of 250 mg/kg, was reported to mitigate the genotoxicity induced by gamma radiation, when administered 1 h before gamma radiation. Citrus extract protected mice bone marrow 2.2-fold, compared to the non-drug-treated irradiated control.<sup>26</sup>

The radio-protective effect of hawthorn (*Crataegus microphylla*) fruit extract was investigated in cultured blood lymphocytes of human volunteers exposed *in vitro* to 150 cGy of gamma radiation. Results suggest that it may be possible to use hawthorn extracts in personnel exposed to radiation in order to protect lymphocytes from the damaging effects of radiation.<sup>28</sup>

The doses of herbal preparations that were effective in protecting against irradiation was significantly lower than the toxic dose of synthetic compounds, and this is one of the major advantages of these agents compared to synthetic compounds.<sup>13</sup> The use of plants as radio-protectors has some disadvantages, such as low to mild efficacy and a short protective time-window (in most cases 30 min to 2 h before irradiation).<sup>13</sup>

## Panax ginseng

Since the 1980s, the radio-protective effects of both *Panax ginseng* (*P. ginseng*) and its partially purified constituents have been demonstrated in experimental tests.<sup>29-38</sup> The strong free radical scavenging effects of *P. ginseng* have been extensively reported.<sup>39-46</sup> Studies have

**Table 1. Radiation sickness.**

System effected/syndrome	Symptoms	Dose
Nervous system CNS or cerebrovascular syndrome	Shock, severe nausea, disorientation, seizures, coma	10,000,000 mrem
G.I. system Gastrointestinal syndrome	Nausea, vomiting, diarrhea, dehydration	1,000,000 mrem
Blood cells /bone marrow Hematopoietic syndrome	Chills, fatigue, hemorrhage, ulceration, infections, anemia	300,000-800,000 mrem
Skin Erythema	Burning/infection, sloughing of skin, hair loss	1,000,000 mrem
Ovaries/testes 250,000-600,000 mrem	Sterility	60,000-80,000 mrem/

CNS, central nervous system; G.I., gastrointestinal.

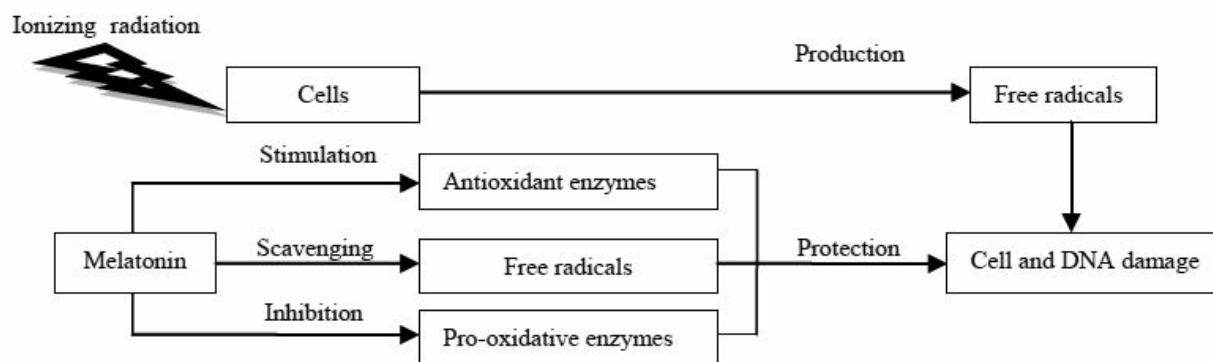


Figure 1. Radio-protective mechanism of melatonin.

shown that ginseng root extracts present both lipid-soluble and water-soluble antioxidant activity, and that this antioxidant action occurs both directly through free radical scavenging and indirectly through upregulation of antioxidant enzymes.<sup>39,40,42,44,46</sup> leading to the prevention of DNA degradation.<sup>39,40,45,46</sup> Kumar *et al.*<sup>47</sup> reported that administration of *P. ginseng* root extract before radiation significantly decreased lipid peroxidation levels and reduced the radiation damage in mice testes. Furthermore, studies have demonstrated the immunomodulating effects of *P. ginseng* in different irradiated animal models. Hsu *et al.*<sup>48</sup> have found that, in mice, the administration of *P. ginseng* before whole body radiation significantly protected both bone marrow stem cells and peripheral hematocytes, thereby reducing the magnitude of radiation-induced regression in the immunohematopoietic system.

## Melatonin: another remarkable radio-protector

Melatonin (N-acetyl-5-methoxytryptamine), a pineal gland hormone involved in regulating the neuroendocrine axis, is a highly efficient free-radical scavenger and antioxidant.<sup>7,49-51</sup> Melatonin has been reported to be a direct free-radical scavenger and an indirect antioxidant via its stimulative effects on antioxidant enzymes,<sup>6,52-54</sup> such as SOD, GSH-Px, glutathione reductase, and catalase<sup>55-59</sup> (Figure 1). Melatonin also increases intracellular GSH levels by stimulating the synthesis of the rate-limiting enzyme,  $\gamma$ -glutamylcysteine synthase, which inhibits the pero-oxidative enzymes nitric oxide synthase and lipoxygenase<sup>55,60,61</sup> (Figure 1).

A large number of studies have indicated the protective effects of melatonin against oxidative stress induced by irradiation.<sup>55</sup> Radiation myelopathy (RM) is known to be one of the most important complications of radiation in clinical radiotherapy and studies have exhibited dose and time dependent radiation effects. Destructive changes in the white matter infrastructure constitute the main histopathological event.<sup>62,63</sup> We have previously assessed the radio-protective effects of melatonin on biochemical, histopathological, and clinical manifestations of RM in rat cervical spinal cord. Administration of melatonin significantly decreased malondialdehyde (MDA) and increased GSH levels when compared with the control group.<sup>60,64</sup> Furthermore, our new data obtained from another study showed that radiation exposure decreased levels of GSH and increased levels of MDA in rat lenses, but these values were within normal limits when melatonin was administered.<sup>65</sup> It was shown that melatonin decreased the formation of 8-hydroxy-2-deoxyguanosine, a damaged DNA product caused by free radicals, 60 to 70 times more effectively than some classic antioxidants (ascorbate

and  $\alpha$ -tocopherol).<sup>66</sup> Thus, melatonin acts as a direct scavenger of free radicals and an indirect stimulator of the activity of the antioxidative defense systems<sup>66-69</sup> (Figure 1).

In another study, Shirazi *et al.*<sup>70</sup> examined the role of melatonin to reduce radiation-induced apoptosis in rat cervical spinal cord. Results from this study suggest that melatonin has protective effects against radiation-induced apoptosis. The principal finding in this work is that melatonin increased *Bcl-2* gene expression with a significant decrease in *Bax* gene expression in irradiated spinal cord. The role of melatonin as an anti-apoptotic agent in healthy cells is a new field of investigation, and information is still limited.<sup>70</sup>

## Conclusions

Research into radio-protective agents must continue in order to find novel radio-protectors that can protect against radiation-induced damage in cells and tissues. Although radio-protectors may be able to minimize the life-threatening effects of irradiation, few radio-protective agents are in clinical use due to undesirable side effects, such as hypotension, vomiting, nausea, sneezing, hot flashes, etc. Therefore, the development of antioxidant radio-protectors with less toxicity are essential areas of radiobiological research as methods of improving the therapeutic index in treatment of patients who have received radiotherapy or other kinds of radiation application.

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