

# Vitamin E Analogs as Radiation Countermeasures: Beyond the Antioxidant Activities

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Since World War II, human beings are at increased risk of Ionizing Radiation (IR) exposure. Proliferation of nuclear weapons and terrorist activities has further intensified the risks of IR exposure to military, civilian, and emergency responders. IR possesses sufficient energy to strip electrons from atoms or molecules and thereby create highly reactive ions. IR at high enough doses induces ionization events that damage DNA, proteins or membrane lipids, either directly or indirectly through intracellular generation of Reactive Oxygen Species (ROS) and other free radicals [1]. Total Body Irradiation (TBI) is especially dangerous if exposed over a short period of time. TBI results in a potentially fatal illness also known as Acute Radiation Syndrome (ARS). Based on the amount of IR absorbed by the body, ARS is characterized as: (a) hematopoietic syndrome (approximately 1–8 Gy), (b) Gastrointestinal (GI) syndrome (8–12 Gy), and (c) cerebrovascular syndrome (>12 Gy) [2].

Given present world scenarios, serious public health concern of IR exposure has justified substantial efforts to develop effective radiation countermeasures. Radiation countermeasures can be broadly classified as: (a) radio protectants (which prevent IR-induced cellular and molecular damage and enhance radiation tolerance), (b) radiation mitigators (which minimize the risk of clonogenic death of normal cells and accelerate recovery or repair after radiation injury), and (c) therapeutic agents (which ameliorate the pathophysiology of IR injury and facilitate tissue recovery and regeneration). The ideal radiation countermeasures should provide significant protection against the acute effects of IR, have minimum toxicity profile, cover a large protective time-window, protect the majority of organs, have an acceptable route of administration, and should have compatibility with the wide range of other drugs that patients may be given during the treatment. Unfortunately, no radiation countermeasure fulfills all of these criteria to date. Over the past half century of research, the United States Food and Drug Administration (FDA) has approved only the synthetic thiol compound amifostine for human use. However, the use of amifostine is greatly restricted by adverse side effects [3]. Therefore, the need for non-toxic and effective radiation counter measures has remained largely unmet.

IR causes the production of large quantity of intracellular ROS through radiolysis of water. Therefore, exogenously added antioxidants or agents that can enhance the activity of endogenous antioxidants remained the focus of investigation for their capacity to ameliorate the deleterious effects of IR in normal tissues [4]. Vitamin E has the well-established health benefits, mediated through its inherent antioxidant, neuroprotective, and anti-inflammatory properties [5]. The Vitamin E family comprises a set of related tocopherols and tocotrienols collectively called tocopherols. The naturally occurring tocopherols encompass  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol, and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienol. Tocopherols and their derivatives have been extensively investigated as radiation countermeasure agents [6]. Their inherent antioxidant properties, ability to scavenge free radicals, lack of performance-degrading toxicity, and ability to suppress chronic radiation-induced fibrosis in some organ systems make them a good candidate for radiation countermeasure development.

Different tocopherols have been extensively evaluated for their radioprotective efficacy in recent years as reviewed by Singh et al. [6]. It

was observed that tocopherols or its derivatives can provide a modest degree of protection against ionizing radiation injuries with dose reduction factor (DRF) ranging from 1.06 to 1.23. By comparison, the FDA-approved amifostine has a DRF 2.7. Sub-cutaneous injection of tocopherols either 1 hour before (DRF 1.06) or 15 min after (DRF 1.1) irradiation significantly increased the 30 day survival of mice [7]. Alpha-tocopherol (AT) enhanced the protection of mice when administered 24 h before  $^{60}\text{Co}$   $\gamma$ -irradiation [8]. The radio-mitigative potential of AT was mediated through the modulation of cell-mediated immunity [9]. Administration of AT after irradiation increased the number of hematopoietic colony-forming units in the spleen of mice [10]. A water-soluble derivative of alpha-tocopherol, known as  $\alpha$ -Tocopherol-Mono-Glucoside (TMG), was demonstrated to have better antioxidant activity [11]; and was found to protect DNA from radiation-induced strand breaks. TMG has been reported to decrease the radiation-induced aberrant metaphases and erythrocytes micronuclei in murine model [12]. TMG was also found effective in preventing radiation-induced bone marrow damage in mice [13].

The hemisuccinate ester derivative of alpha-tocopherol also known as alpha-Tocopherol Succinate (TS) is the most effective form of vitamin E for adjuvant cancer treatment [14]. Additionally, TS enhanced the radiation-induced chromosomal damage in cancer cells selectively rendering normal cells unaffected *in vivo* [15]. Our recent studies with TS demonstrate that, TS when administered 24 h before radiation exposure enhanced the survival of mice irradiated with gastrointestinal dose  $^{60}\text{Co}$   $\gamma$ -radiation. TS rendered radioprotection to mice by protecting the intestinal tissue of irradiated mice by maintaining the number of crypt, villi, and mitotic figures. TS protected the radiation-induced GI damage by inhibiting apoptosis, promoting regeneration of crypt cells, and inhibiting translocation of gut bacteria. We observed that TS could protect lethally irradiated mice by inhibiting radiation-induced apoptosis and DNA damage and enhancing cellular proliferation. TS induced very high levels of Granulocyte Colony-Stimulating Factor (G-CSF) and Keratinocyte-Derived Chemokine (KC) in the peripheral blood of mice. We observed that neutralization of G-CSF in circulating blood significantly abrogated the protective effect of TS [16].

Delta Tocotrienol (DT3) having greater antioxidant activity than that of  $\gamma$ - and  $\alpha$ -tocotrienol has also gained attention as a radiation countermeasure. Administration of DT3 before or after irradiation significantly protected mice from lethal radiation. DT3 reduced the radiation-induced apoptosis by inhibiting the activation of caspase-8,

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caspase-3 and caspase-7, while increasing autophagy-related beclin-1 expression in irradiated bone marrow [17]. DT3 has been shown to render radioprotection by stimulating extracellular signal-related kinase (Erk) activation associated with the mammalian target of the rapamycin (mTOR) survival pathway [18]. Another form of tocotrienol, gamma tocotrienol (GT3), has received great attention in recent years. GT3 been reported as a potent inhibitor of 3-Hydroxy-3-Methylglutaryl-Coenzyme A (HMG-CoA) reductase, a regulatory enzyme for cholesterol biosynthesis. GT3 significantly protected mice against lethal dose of radiation. GT3 enhanced the number of colony-forming cells and regenerative microfoci for myeloid and megakaryocytes, while reducing the frequency of micro-nucleated erythrocytes in irradiated mice [19]. GT3 treatment also enhanced the induction of G-CSF in mice, alluding to its possible mechanism of action of GT3 as a radiation countermeasure. We recently observed that neutralization of GT3-induced G-CSF by antibody abrogated the GT3-mediated radiation protection in murine model [20].

Most of the tocols and their derivatives disused here have the potential to be developed as novel radiation countermeasures. However, additional preclinical studies are required to establish the mechanisms of action of these radiation countermeasures. Further knowledge of mechanisms of action of these countermeasures will facilitate to design the combination formulations which may have better efficacy against the lethal dose of ionizing radiation. Berbee and coworkers have shown that the combined treatment of GT3 and pentoxifylline significantly enhanced the survival of irradiated mice compared with the survival of irradiated mice treated with either GT3 or pentoxifylline alone [21]. The GT3 in combination with pentoxifylline could confer greater protection against lethal dose of radiation by improving bone marrow colony forming units, increasing spleen colony counts, and enhancing platelet recovery compared to GT3 alone. We observed that TS induces higher level of G-CSF in mice, and therefore hypothesized that TS could encourage bone marrow progenitor cells mobilization into the peripheral blood. We observed that TS enhanced the mobilization of hematopoietic progenitors from bone marrow to peripheral blood [22]. Further, we observed that infusion of whole blood or PBMC collected from TS- and AMD3100-injected mice significantly improved survival of mice receiving still higher GI radiation doses [23]. We further observed that the infusion of PBMC from TS- and AMD 3100-injected mice significantly inhibited apoptosis, increased cell proliferation in the analyzed tissues, and inhibited gut bacterial translocation to various organs [24]. These studies further support our hypothesis that TS could be utilized as a mobilizer of progenitor cells and infusion of PBMC containing TS-mobilized progenitor cells could be used as a bridging therapy for radiation casualties. Here we propose an innovative way to treat individuals (military personnel or first responders) who are at high risk of acute IR exposure. Military personnel, preparing for special missions, may be treated with TS to mobilize their own progenitor cells; and blood samples of these individuals may be stored frozen before starting the mission. In the event of a radiation exposure, the person could receive a therapeutic transfusion of their previously stored blood/PBMC containing a higher number of progenitor cells.

These and other investigations into the vitamin E and their derivatives as novel radiation countermeasures, including their mechanisms of action, have significantly advanced over last decade. However, extensive research is still required to investigate the mechanisms of action at the molecular level. Only limited reports for the identification and development of efficacy biomarkers for these drugs are available. As most of these observations are based on experiments

conducted in the murine model, studies using non-human primate models are needed. Nevertheless, as shown in the research reports above, vitamin E and its derivatives have shown significant potential as novel and effective radiation countermeasures.

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