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Gamma tocotrienol, a potent radioprotector, preferentially upregulates expression of anti-apoptotic genes to promote intestinal cell survival

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ABSTRACT

Gamma tocotrienol (GT3) has been reported as a potent ameliorator of radiation-induced gastrointestinal (GI) toxicity when administered prophylactically. This study aimed to evaluate the role of GT3 mediated pro- and anti-apoptotic gene regulation in protecting mice from radiation-induced GI damage.

Male 10- to 12-weeks-old CD2F1 mice were administered with a single dose of 200 mg/kg of GT3 or equal volume of vehicle (5% Tween-80) 24 h before exposure to 11 Gy of whole-body γ-radiation. Mouse jejunum was surgically removed 4 and 24 h after radiation exposure, and was used for PCR array, histology, immunohistochemistry, and immunoblot analysis.

Results were compared among vehicle pre-treated no radiation, vehicle pre-treated irradiated, and GT3 pre-treated irradiated groups. GT3 pretreated irradiated groups, both 4 h and 24 h after radiation, showed greater upregulation of anti-apoptotic gene expression than vehicle pretreated irradiated groups. TUNEL staining and intestinal crypt analysis showed protection of jejunum after GT3 pre-treatment and immunoblot results were supportive of PCR data.

Our study demonstrated that GT3-mediated protection of intestinal cells from a GI-toxic dose of radiation occurred via upregulation of antiapoptotic and downregulation of pro-apoptotic factors, both at the transcript as well as at the protein levels.

1. Introduction

Changing world political scenarios along with use of nuclear technology to meet our increasing energy needs pose risk of exposure to acute high doses of radiation resulting either from terrorist activities or from accidents at nuclear facilities. Radiation exposure – depending on dose, duration of exposure, and body area exposed – is associated with a risk of developing incapacitating pathophysiological changes in a number of organs critical for survival (Andrews, 1967; Hall and Giaccia, 2006; Citrin et al., 2010). Sudden exposure to a large dose of radiation is associated with development of the acute radiation syndrome (ARS), which, depending on radiation doses could involve the hematopoietic, gastrointestinal (GI), or neurovascular systems (Andrews, 1967; Hall and Giaccia, 2006; Citrin et al., 2010). Exposure to radiation doses above 8 Gy typically develops GI syndrome and doses above 20 Gy predominantly develops neurovascular syndrome. While fatalities could occur within hours after exposure to doses above 20 Gy due to neurovascular toxicity, prophylactic administration of medical countermeasure agents, alone or in combination, could make a difference between patient survival and death at lower doses (Zenk, 2007; Coleman and Parker, 2009). Efforts are on for the last several decades to develop agents that when administered prophylactically could ameliorate ARS in first responders, emergency workers, and the civilian population at risk following a radiological event (Whitnall et al., 2002; Landauer et al., 2003;

Abbreviations: GT3, γ-tocotrienol; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; GI, gastrointestinal; ARS, acute radiation syndrome; PARP, poly-ADP-ribose polymerase; ATM, ataxia-telangiectasia-mutated; DNA-PK, DNA-dependent protein kinase; ROS, reactive oxygen species; DRF, dose reduction factor.

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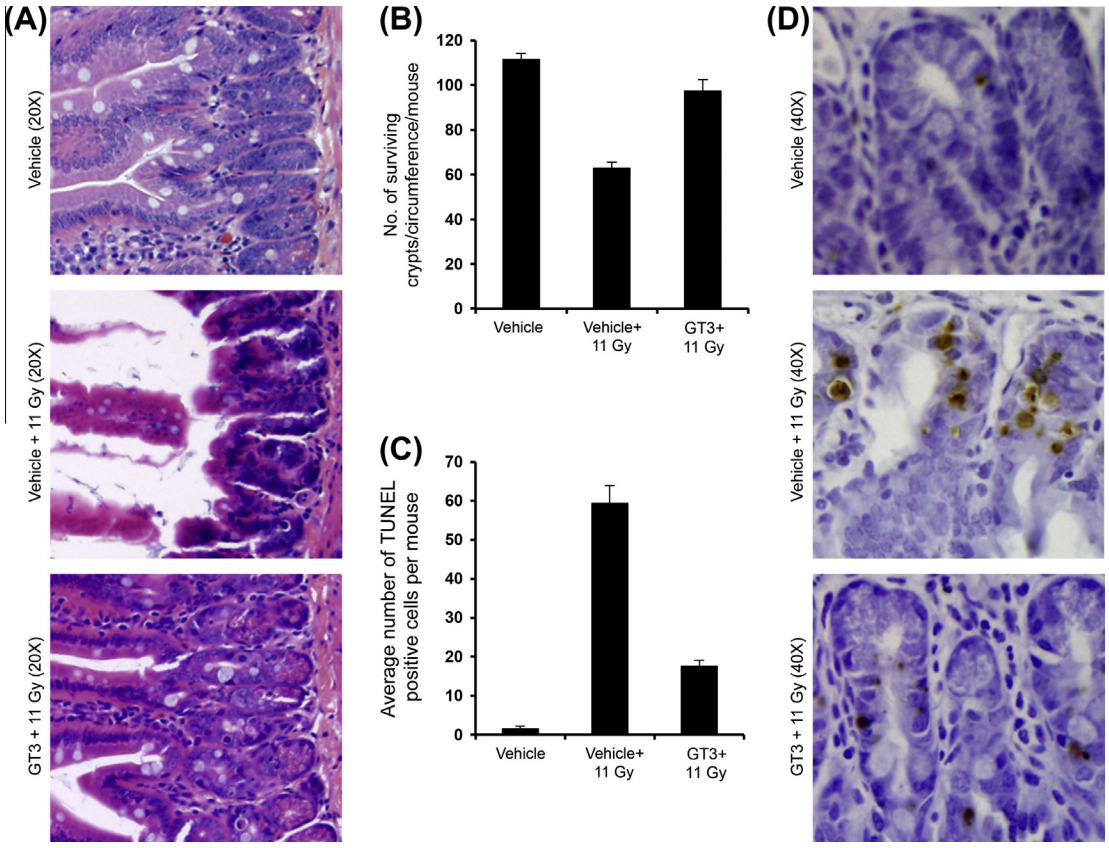

Fig. 1. GT3 pre-treatment supported crypt integrity and cell survival after radiation exposure. (A) H&E stained representative images (20×) of intestinal sections showing greater crypt-villi structural integrity in GT3-treated mice. (B) Quantification of surviving crypts showed higher survival in GT3 pre-treated mice relative to vehicle pre-treated groups. (C) TUNEL staining images (40×) showed GT3 pre-treatment reduced radiation-induced apoptosis of intestinal cells. (D) Quantification of TUNEL positive cells showed significantly reduced number in GT3 pre-treated irradiated mice relative to vehicle pre-treated irradiated groups. Data presented as mean ± standard error of mean (SEM) and $p < 0.05$ was considered significant.

Pamujula et al., 2005; Yildiz et al., 2006; Suman et al., 2012a, 2012b). Prophylactic intervention has potential for protecting intestinal cells from radiation-induced GI-toxicity. Several agents, including cytokines such as interleukin-11 (IL-11), have shown promising survival advantage in experimental animals after exposure to GI toxic radiation doses (Potten, 1995; Weiss et al., 1995; Wang et al., 1999; Berbee et al., 2009, 2012). However, we are yet to develop a safe, effective, and FDA approved radioprotectant that could be used in a radiological event. Therefore, development of prophylactic radiation countermeasure agents is a high-priority research area.

Although a wide range of synthetic and natural compounds have been screened for their radioprotective properties (Citrin et al., 2010; Singh et al., 2012; Suman et al., 2012a, 2012b), toxicity remains a major concern for developing synthetic radioprotectors, consequently limiting their human use (Giambarresi and Walker, 1989). To this end, natural products and vitamin derivatives with their known safety status and proven beneficial effects in humans are being considered actively over synthetic compounds for developing radioprotectors. The tocol family of compounds, which includes vitamin E, is known for strong antioxidant properties and consists of eight compounds. While tocopherols are tocols with saturated side chains having α, β, γ and δ isoforms, tocotrienols also are tocols with the same four isoforms (α, β, γ and δ) yet unlike tocopherols, tocotrienols have unsaturated side chains (Cook-Mills and McCary, 2010). Both tocopherols and tocotrienols have been shown to be relatively non-toxic even at higher doses (Singh et al., 2010, 2011, 2012), and were shown to provide significant survival advantage from radiation toxicity in mice (Ghosh et al., 2009b; Singh et al., 2012). Among tocols, γ-tocotrienol (GT3) has been shown to have an effective antioxidant property with higher reactive oxygen species (ROS)-quenching potential. GT3 also provided higher radioprotection than other tocol family compounds (Ghosh et al., 2009b; Singh et al., 2012). Furthermore, GT3 has unique side chain arrangements that have been proposed as a possible mechanism of its higher protective properties (Ghosh et al., 2009a).

GT3 (200 mg/kg) when administered prophylactically has been shown to provide significant survival advantage after exposure to 11 Gy γ-radiation, a GI-toxic dose (Ghosh et al., 2009b). Furthermore, GT3 demonstrated a radiation dose reduction factor (DRF) of 1.29 after a single 200 mg/kg dose administered subcutaneously in mice – higher than α-tocopherol (Ghosh et al., 2009b). Importantly, a single dose of 400 mg/kg of GT3 administered 24 h before radiation protected intestinal mucosal surface area as well as improving post-radiation survival (Berbee et al., 2009; Berbee and Hauer-Jensen, 2012). The protective effects of GT3 also have been reported in different cell types including renal cells, endothelial cells, and hematopoietic cells (Berbee et al., 2009, 2011, 2012; Kulkarni et al., 2010, 2012; Nowak et al., 2012). Apart from its antioxidant effects, GT3 has been proposed to mediate at least in part its radioprotective effects by 3-hydroxyl-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase inhibition, DNA damage prevention, increased cytokine production, differential gene expression, mitochondrial protection, and by maintaining cellular tetrahydrobiopterin levels (Berbee et al., 2009, 2011, 2012; Kulkarni et al., 2010, 2012; Nowak et al., 2012). Furthermore, a number of studies have used comet assay to report that both tocopherols and tocotrienols