IR-induced DNA damage is reduced in normal fibroblasts and enhanced in cancer cells by gammatocotrienol

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Gamma-tocotrienol (GT3) is an isoform of vitamin E with high anti-oxidant activity. Studies by us have demonstrated that GT3 has low-toxicity and protects mice from lethal irradiation. A study on a limited number of mice has indicated that protective activity of GT3 is selective towards normal tissues, while towards tumors it acts as a radio-sensitizer. The mechanism of selectivity is still unknown. It has been proposed that GT3 accumulates in the tumor as a pro-oxidant quinone and enhances radiation-initiated oxidative stress while in normal cells GT3 act as an anti-oxidant. Oxidative stress from radiation results in DNA-damage and cell death. To study the mechanisms of differential response of GT3 to cancer and normal cells, we used MCF-7 breast cancer cells and AG01522 normal cells incubated in vitro with GT3 and irradiated. Following radiation, these cells were used to determine whether the presence of GT3 reflects in differential levels of DNA-damage. Material and methods: AG01522 normal fibroblasts and MCF-7 breast cancer cells were used for this study. Toxicity and proliferation were measured with the WST-1 assay. Viability was measured with CitoTox-One assay and Trypan Blue exclusion method. γ-H2AX immunostain was used to assess DNA-damage. Irradiation was done using a Co source (2 Gy, 0.6 Gy/min). Survival was determined by clonogenic assay. Results: Most radio-protectors/sensitizers fail in the clinical settings because of their high toxicity. GT3 exhibits levels of toxicity which are cell type dependent. AG01522 were more sensitive to GT3-induced toxicity than MCF-7, but were viable up to 5 ug/ml. Safety of the dose was confirmed by proliferation, viability and cell cycle distribution analysis. To determine radiation-induced DNA-damage in GT3-treated cells, we examined phosphorylation of H2AX (γ-H2AX) at various time points after irradiation by confocal microscopy. γ-H2AX localizes at sites of DNA double-strand-breaks; levels of γ-H2AX and kinetics of removal have been associated with DNA-repair and survival. GT3 reduced the number of γ-H2AX foci in AG01522 and increased the surviving fraction, while it increased DNA-damage and reduced survival in MCF7. Phosphorylation of H2AX was delayed and persistent in GT3-treated MCF-7 cells. Conclusion: In vitro irradiation of GT3-treated normal and cancer cells indicate that GT3 has selective radio-protective/sensitizing roles. Radio-protection of normal cells was associated with reduced DNA-damage, while radio-sensitization of cancer cells was associated with increased DNA-damage and persistent levels of γ-H2AX foci, suggesting a role for GT3 in differential modulation of DNA-repair.

Article published by EDP Sciences and available at http://www.radioprotection.org or http://dx.doi.org/10.1051/radiopro:2008687