Impact of Topical Deferoxamine Used as a Radioprotective Agent on the Efficacy of Radiation Therapy in a Murine Model of Human Breast Cancer

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INTRODUCTION

Transdermal deferoxamine patch (DFO) is a novel experimental treatment with potential to reduce radiation-induced skin injury in breast cancer patients undergoing radiation. While the radioprotective effects of DFO patches on skin have been established, the potential impact of DFO application on the efficacy of radiation therapy remains unexplored.

Goal: Therefore, this study investigates the impact of topical DFO treatment on the efficacy of radiation therapy.

STUDY DESIGN

Animal model: Human breast cancer in a xenograft murine model
Cancer cell line: MCF7-luc

(A)

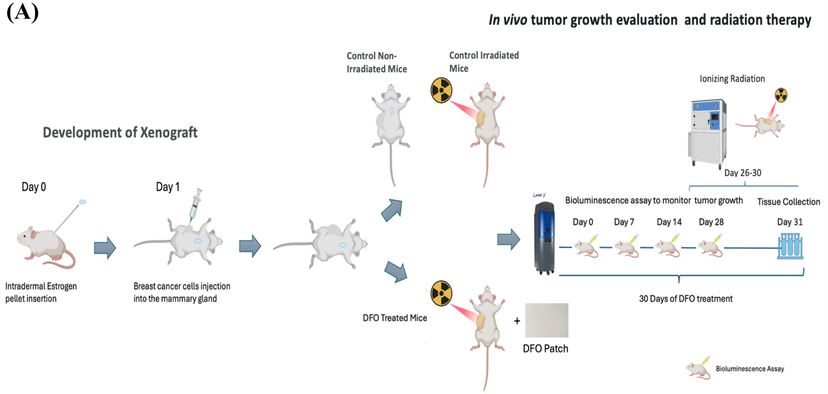

Figure 1. Outline of experimental design.

Study design: The study used a murine model of human breast cancer, injecting 1x10^6 MCF7-Luc2 cancer cells into the mammary gland of 8-week-old female immunodeficient NSG mice. Once tumors reached an intensity of ~1x108 photons/s, mice were divided into three cohorts: control non-radiated, control irradiated and irradiated DFO-treated. DFO was applied daily for 30 days until the study endpoint. Radiation therapy (5 x 2 Gy daily; total 10 Gy) was administered on the last 5 days of DFO treatment using the RS-2000 irradiator. Pre- and post-radiation tumor size was assessed using luciferin injection and LAGO bioluminescence imaging. Skin and tumor samples were preserved in formalin and embedded in paraffin for analyses. Immunofluorescence staining, TUNEL staining and ImageJ software were used to quantify blood vessel, cell proliferation and apoptotic cells. Statistical analysis was performed using ANOVA and GraphPad Prism, with $p < 0.05$ considered significant.

RESULTS

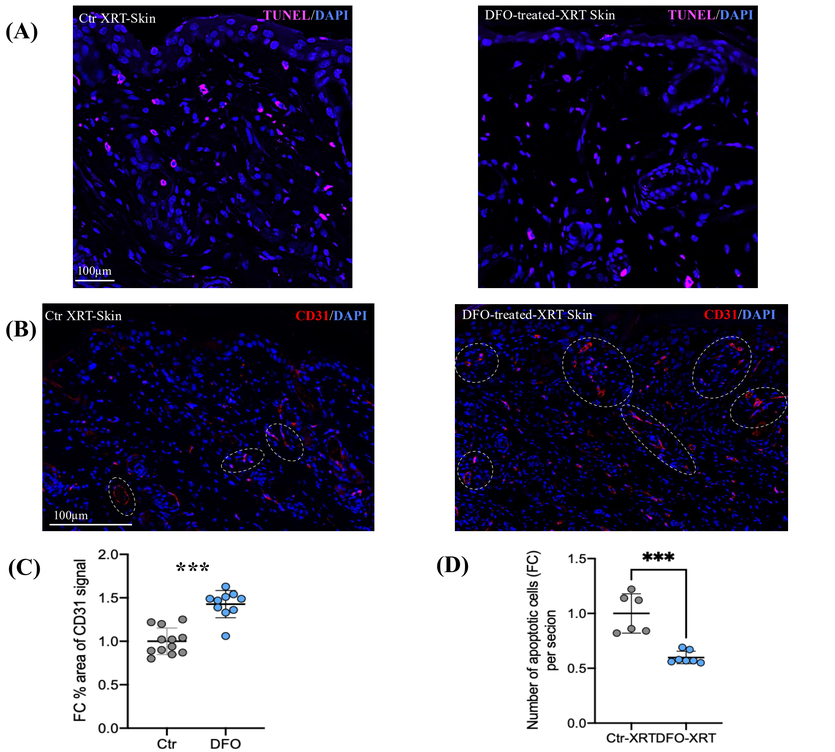

Figure 2. Quantitative analysis for IF and TUNEL staining indicated an increase in blood vessel formation (CD31) in irradiated DFO-treated skin compared to controls, and a decrease in number of dead cells in irradiated DFO-treated skin compared to controls confirming successful topical DFO treatment. (A) Representative images of TUNEL staining for control and DFO-treated skin. **(B)** Representative images of IF staining of CD31 for control and DFO-treated skin. **(C)** Quantitative analysis for CD31 for control and DFO treated skin. **(D)** Quantitative analysis of apoptotic cells for control and DFO treated skin.

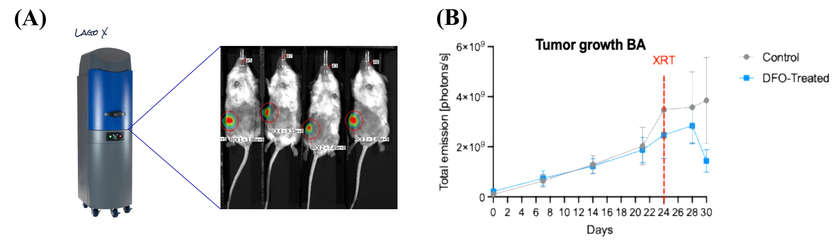

Figure 3. Bioluminescence assay, using Lago imaging showed no difference in tumor growth over the period of 30 days between irradiated DFO-treated and control tumors, indicating that topical DFO does not affect tumor growth. (A) Representative image of Bioluminescence assay via Lago X imaging system. **(B)** Graph representing relative tumor growth between irradiated DFO treated and control tumor over a period of 30 days.

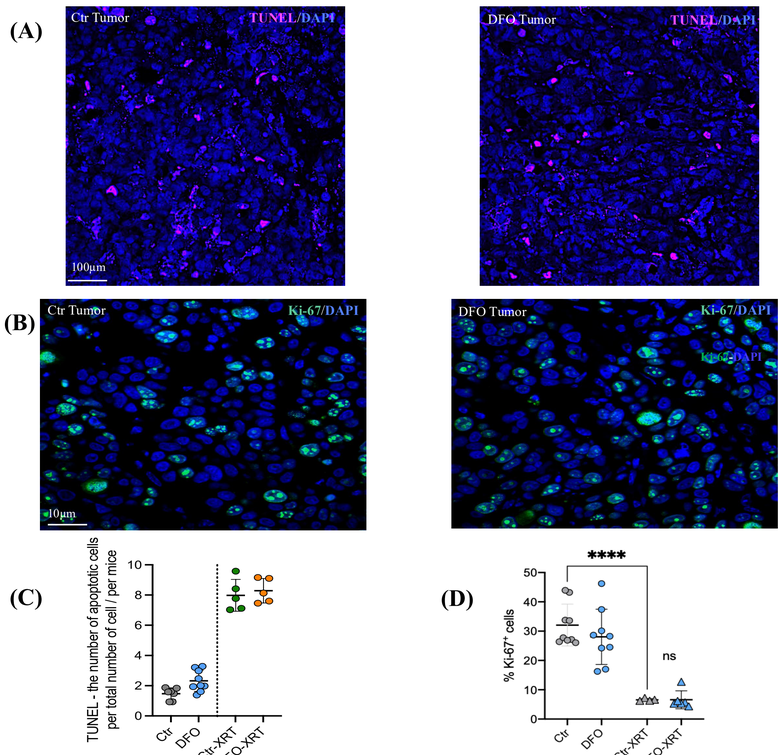

Figure 4. Quantitative analysis of TUNEL and IF staining for Ki-67 showed no significant changes in cancer cell death and cancer cell proliferation respectively, between irradiated untreated tumors compared to irradiated DFO-treated group, but both irradiated groups had significantly higher dead cells and lower proliferative cells, respectively when compared to non-irradiated controls, indicating that topical DFO does not interfere with radiation therapy. (A) Representative images of TUNEL staining for control and DFO-treated tumors. **(B)** Representative images of IF staining for Ki-67 in controls and DFO treated tumors. **(C)** Quantitative analysis of apoptotic cells for control and DFO treated tumors. **(D)** Quantitative analysis of the number of Ki-67 positive cells for controls and DFO treated tumors.

CONCLUSIONS

- ❑ Improved skin vascularization observed on the skin of DFO-treated mice following irradiation.
- ❑ Topical DFO does not limit radiation efficacy as shown by inhibited tumor growth after radiation was administered.
- ❑ Therefore, we hypostatize that lack of DFO effect on cancer cells is due to limited ability of DFO patch to penetrate deeper tissue or limited to the skin and does not interfere with the radiation therapy on tumor cells.

Our study suggests that DFO does not interfere with the irradiation therapy on breast cancer cells and can be safely used a s a radioprotective treatment.