Topical Application of GS-Nitroxide JP4-039 Emulsion Mitigates Ionizing Irradiation Induced Skin Burns

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Abstract

Objectives: Exposure to high doses of ionizing electron beam irradiation results in a significant skin burn. There is a need to design mitigating agents to reduce ionizing irradiation-induced skin injury. We have developed an emulsion (F14) containing a systemic total body irradiation mitigator, mitochondrial targeted 4-amino Tempol (JP4-039). In a mouse model we sought to demonstrate that application of the emulsion to the skin following 30 or 35 Gy single fraction electron beam irradiation mitigates radiation damage.

Materials/Methods: An F14 emulsion consisting of sesame seed oil, phosphatidyl choline, saline and JP4-039 (4 mg/ml) has been developed (F14-JP4-039). The right legs of C57BL/NJsd mice were shaved and treated with Nair to remove the hair. Twenty-four hours later the right legs were irradiated to doses of 30 or 35 Gy using 6 MeV electron beam, biologically to bring the surface dose to 100%. At 30 minutes after irradiation, 24, 48 and 72 hours, F14 emulsion only or F14-JP4-039 (100 µg in 50 µl) was applied to the skin. Photos were taken for visual evidence of skin damage and leg contracture was measured as a functional evaluation of tissue damage. Histological characterization of skin thickening, cellular infiltrates and collagen evaluation was also performed. To evaluate the mechanism of radiation damage mitigation, skin samples were sacrificed at 4 hours after irradiation for markers of apoptosis and oxidative stress.

Results: Skin damage was scored on day 21 after irradiation. Damage after 35 Gy with F14-JP4-039 (15.2 ± 0.2) was significantly reduced compared to control (19.5 ± 0.9, p < 0.01) and F14 emulsion treated (18.3 ± 0.4, p < 0.01). There was also a significant decrease in dermal thickness (2.3 ± 0.1 in F14-JP4-039, 3.3 ± 0.2 in control, 3.8 ± 0.2 in F14 emulsion, p < 0.05). These results were consistent with increases in collagen levels observed with Masson's Trichrome staining. The F14-JP4-039 treated skin showed less inflammation as evidenced by reduced cellular infiltrates observed with H&E staining (3.2 ± 0.3) compared to control irradiated skin (4.6 ± 0.2, p < 0.05) and F14 treated irradiated skin (5.0 ± 2.8, p < 0.05). There was also a significantly decreased apoptosis 4 hours after irradiation in skin treated with F14-JP4-039 (15.5 ± 4.2% of cells) compared to the control and F14 treated mice (35.0 ± 5.8 or 24.2 ± 4.2%, respectively, p < 0.05). The skin on the right leg was then removed and cut into three sections. One was frozen on dry ice, one section fixed in formalin and one section frozen in OCT (Optimum Cutting Temperature). Histological sections were characterized by skin thickening, cellular infiltrates and collagen evaluation. The mechanism of mitigation of irradiated skin damage was performed by analysis of apoptosis and oxidative stress.

Conclusions: The skin damage was measured using visual evidence seen from photographs of the skin and measuring leg contracture. The skin on the right leg was then removed and cut into three sections. One was frozen on dry ice, one section fixed in formalin and one section frozen in OCT (Optimum Cutting Temperature). Histological sections were characterized by skin thickening, cellular infiltrates and collagen evaluation. The mechanism of mitigation of irradiated skin damage was performed by analysis of apoptosis and oxidative stress.

Figure 1: Skin damage following 35 Gy irradiation. Fat was removed from the right rear leg of female C57BL/NJsd female mice. The mice were irradiated to 35 Gy, and treated with F14 emulsion or F14 emulsion containing JP4-039. Mice treated with F14-JP4-039 had less skin damage than irradiated mice only or irradiated mice treated with F14.

Figure 2: F14-JP4-039 application following irradiation mitigates irradiation skin damage. Mice which had been irradiated 21 days before to either 30 or 35 Gy were sacrificed, and the skin was analyzed for irradiation damage. The skin was stained for apoptosis and oxidative stress. The skin of C57BL/6NHsd was irradiated to 35 Gy and treated with F14-JP4-039. The skin was also stained for apoptosis 4 hours after irradiation in skin treated with F14-JP4-039 (15.5 ± 4.2% of cells) compared to the control and F14 treated mice (35.0 ± 5.8 or 24.2 ± 4.2%, respectively, p < 0.05).

Figure 3: Application of F14-JP4-039 results in decreased cellular infiltrates and dermal thickness in mice irradiated 35 Gy + F14-JP4-039 compared to 35 Gy only or 35 Gy + F14 emulsion. Mice treated with F14-JP4-039 had less skin damage than irradiated mice only or irradiated mice treated with F14 emulsion (A). In addition, the differential leg extension was used as an indicator of skin damage with a larger extension as an indicator of increased damage (B). The skin treated with F14-JP4-039 had a significant decrease in differential leg extension compared to the irradiated skin or irradiated skin treated with F14 emulsion only.

Figure 4: Application of F14-JP4-039 after irradiation results in decreased apoptosis. Application of a F14 emulsion containing JP4-039 at 30 min after irradiation resulted in a significant decrease in the number of apoptotic cells compared to control irradiated skin (30 Gy alone) (11.3 ± 3.2 %). H&E stained sections of skin irradiated to 35 Gy resulted in an increase in thickening of the skin which was significantly reduced by treatment with F14-JP4-039 (B).