The GS-nitroxide JP04-39 is a Potent Topical Antioxidant that Can Mitigate Skin Damage from Ionizing Radiation

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BACKGROUND

- Cutaneous damage from radiation exposure is a therapeutic challenge.
- There is a need for protective (delivered before radiation exposure) and mitigating agents (delivered after irradiation) specific for ionizing irradiation induced cutaneous injury.
- Mitochondrial damage is critical in initiation of ionizing irradiation-induced cellular apoptosis, tissue injury, and organ failure.
- The designed radioprotective and mitigating agents capable of stabilization of mitochondrial membranes is a rational approach to damage prevention/therapy.
- The GS-nitroxide JP04-39 (Figure 1) is a hemigramicidin nitroxide antioxidant capable of mitigating radiation toxicity.

METHODS

- Mouse flank was shaved and depilated 24 hrs prior to irradiation with 35 GY using a 6 MeV electron beam.
- Clinical Effect- 21 Day Experiments
  - JP4-039 (Figure 1) was topically applied in a liposome formulation 30 minutes after irradiation and daily for the next 3 days.
  - Mice were photographed and skin damage scored by clinical criteria.
  - Leg contracture was measured by comparing maximal extension between control and irradiated legs.
  - Histological characterization of cellular infiltrates and collagen levels
- Mechanism- 4 Hour Studies
  - JP4-039 (Figure 1) was topically applied in a liposome formulation 30 minutes after irradiation. Skin samples taken at 4 hours.
  - Analyzed for apoptosis via caspase 3 and TUNEL
  - antioxidant functions evaluated by changes in GSH levels.
- All quantitative data presented as mean ± sem and significance determined by ANOVA followed by a Bonferroni post test at p<0.05.

OBJECTIVE

Demonstrate that topical delivery of the novel mitochondrial targeted small molecule antioxidant JP04-39 will mitigate ionizing irradiation induced skin damage.

PRELIMINARY CONCLUSIONS

- Topically applied JP04-39 mitigates clinically evident acute skin damage.
- Damage mitigation correlates with reductions in cellular infiltrates and fibrosis.

Topically applied JP04-39 is a potent antioxidant that can prevent/reverse acute radiation damage.

Figure 1- JP4-039

Figure 2- Clinical changes 21 days after irradiation with 35 GY are improved by JP04-39

0 GY 35 GY
35 GY+ Formulation 35 GY+JP04-39

Damage scores are significantly higher 21 days after irradiation than without irradiation (p<0.001). JP04-39 treatment significantly reduces radiation damage (p<0.001 vs 35 GY alone).

Figure 3- Fibrosis is reduced by JP04-39 as evidenced by improved leg extension.

Irradiation reduces mouse leg stretching capacity, an indication of collagen level and potential fibrosis in vivo. Data are graphed as the difference between full extension in irradiated and non-irradiated legs. JP04-39 significantly reduces damage (p<0.01).

Figure 4- Radiation- induced cellular infiltrates are decreased by JP04-39 21 days after irradiation with 35 GY.

0 GY 35 GY 35 GY+ Form 35 GY+JP

H&E sections demonstrate that radiation-induced cellular infiltrates are reduced by JP04-39 (p<0.01 versus 35 GY alone)

Figure 5- JP04-39 reduces apoptosis and GSH depletion 4 hours after irradiation with 35 GY.

JP04-39 reduces irradiation-induced apoptosis measured by caspase activity (p<0.01)(3A) and TUNEL (p<0.05)(3B). Percent apoptotic cells in epidermis were quantitated by counting at 400X.

JP04-39 reverses loss of the antioxidant GSH in irradiated skin (p<0.05 vs irradiation alone)