UNCLASSIFIED

Identification of Endogenous Radioprotective Factors in a Rodent Model of Radiation Combined Injury

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Introduction

In the current geopolitical climate, there is increasing concern for nuclear events endangering civilians and military personnel. Radiation injuries in a battlefield context are expected to be combined with other injuries such as blast trauma, burn, and infection, termed radiation combined injuries (RCI)[1]. To date, all treatments for RCI are supportive in nature, focusing on infection control, wound care, and hematopoietic support rather than directly mitigating the combined effects of radiation and trauma. As such, there is an urgent unmet need for the development of medical countermeasure (MCMs) that can effectively address the complex and synergistic effects of RCIs.

Our institution has developed a rat blast/radiation model, and survival studies show higher survival rates in the RCI group compared to those with radiation exposure alone. We *hypothesize* that RCI activates endogenous radioprotective factors that may stabilize the hematopoietic system.

Objective

The *central objective* of this study is to examine and characterize the radioprotective effects of rat RCI serum on hematopoietic cells in vitro.

Methods

Blood Collection - Blood was collected from anesthetized rats exposed to either blast overpressure (BOP), whole body irradiation (WBI), combined BOP/WBI, or unexposed (CTRL) 7 days post-injury.

Lethality Curves – Rat lymphoblast TIB236 cells were exposed to 0-12 Gy total ionizing radiation. Viability assays were conducted using acridine orange/propidium iodide (AO/PI) to determine the irradiation LD_{50} at 48 hrs .

Serum Exposure Assays - TIB236 cells were exposed to 5 Gy then cultured in media supplemented with 10% serum from each experimental group at 37˚, 5% CO_2. Viability assays were performed at 24, 48, and 72 hrs with AO/PI.

Antibody Arrays – Rat proteome arrays were performed using serum from the four experimental groups. Arrays were imaged and semi-quantitative analysis performed to identify differential expression amongst the groups.

Results

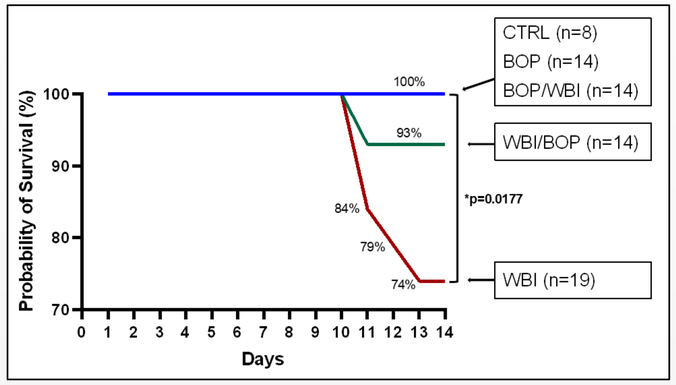

Figure 1: Kaplan-Meier curve depicting probability of 14-day survival after 175 kPa blast overpressure (BOP) and 5.5 Gy whole body irradiation (WBI) exposure in Sprague-Dawley rats. All CTRL, BOP, and BOP/WBI-exposed animals survived until day 14, while 93% of animals that received WBI before BOP survived. Animals that were exposed to WBI had a14-day survival of 74%.

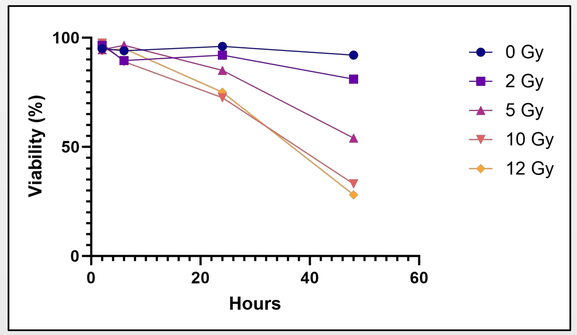

Figure 2: Lethality curve showing percent viability of naïve TIB236 rat lymphoblast cells exposed to 0-12 Gy. Exposure to 5 Gy resulted in ~50% viability (LD_{50}) at 48 hrs.

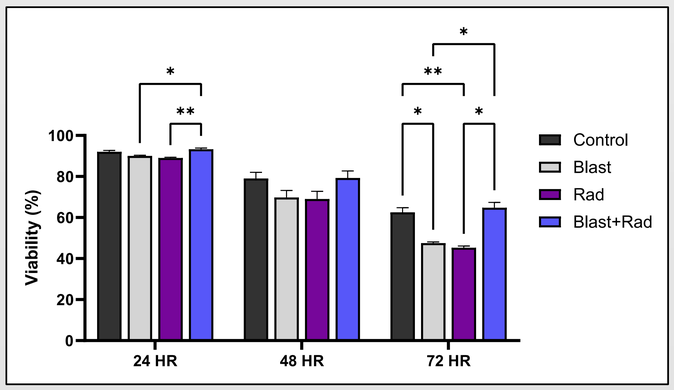

Figure 3: Viability of TIB236 cells exposed to 5 Gy IR followed by treatment with either CTRL, BOP (B), WBI (R), BOP/WBI (B/R) serum. At 72 hrs post-IR, cells treated with CTRL and BOP/WBI serum showed significant increases in viability compared to cells treated with BOP and WBI serum. *p<0.05, **p<0.01

Results

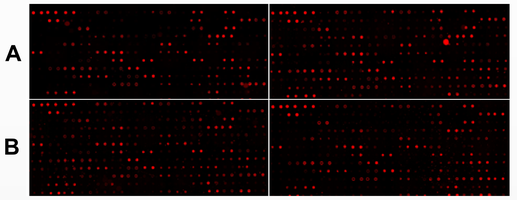

Figure 4: Representative images of rat proteome antibody arrays. CTRL (A), BOP (B), WBI (C), BOP/WBI (D) serum.

Pathways	Proteins	Trauma Role
Cytokine & Immune Signaling	IL-1 F5, IL-1 F9, IL-12 RB1, IL-13, IL-2 R beta, IFNGR1, G-CSF R, SCF R	Inflammation, immune response
Apoptosis	TRAIL R2, CRADD, Granzyme M	Cell death, tissue remodeling
Stress Response	Haptoglobin, S100A11, S-100b, Hepassocin, ProSAAS, PTMA, GSR	Inflammation, neuroprotection, oxidative stress response
Coagulation & Vascular Repair	Factor VII, PF4, VEGFR3	Blood clotting, wound healing, angiogenesis
Cell Cycle/Proliferation	Cyclin D1, PLS3, CCT3, Septin-7, TFF1	Cell proliferation, migration, tissue repair
Leukocyte Migration	L-Selectin	Immune cell recruitment

Table 1: Differential upregulation of serum proteins involved in apoptosis, immune signaling, and stress response was observed in the serum of BOP and WBI animals when compared to CTRL and BOP/WBI animals.

Conclusions

- Rat model of BOP/WBI combined injury showed higher 14-day survival rates than BOP or WBI injury alone.
- Treatment with serum from CTRL and BOP/WBI animals conferred increased viability in a cell model of radiation injury.
- Differential upregulation of inflammatory, apoptotic, and stress proteins was observed in serum of BOP and WBI animals compared to CTRL and BOP/WBI animals.
- More work is necessary to determine if adaptive responses such as hormesis or mechanical stress are responsible for the observed radioprotective benefits.
- Future work will be aimed at identifying candidate targets for development of novel RCI MCMs.

References
1.) Kiang JG, Olabisi AO. "Radiation: a poly-traumatic hit leading to multi-organ injury." Cell Biosci, 2019; 9:25.

Disclaimer
Research was conducted in compliance with Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC International.

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