Novel Protective Effects of BCI on Survival of Male and Female Macrophages after Lethal Radiation Injury: Changes in MAPK Activation, Cytokine Production and miR-34a Expression

Juliann G. Kiang[1,2], Katherine S. Cleveland[1,3], Akeylah K. Woods[1], Min Zhai[1,3], Lavanya Mahajan[1], Armin Tabatabai[1], Chang-Lae Jo[1], Georgetta Cannon[1], and R. Joel Lowy[1]

[1]Radiation Combined Injury Program, Armed Forces Radiobiology Research Institute; [2]Dept. of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD; [3]Henry Jackson Foundation for the Advancement of Military Med, Inc, Bethesda MD

ABSTRACT

Introduction: In response to excessive ionizing radiation exposure, radiation injury (RI) occurs and manifests principally as hemopoietic acute radiation syndrome (ARS), GI ARS and/or cutaneous ARS. Macrophages are present in every organ and tissue, perform important host defense functions to physical injury, and are essential to innate immunity, but how radiation effects on their function are understudied. There are no U.S. Food and Drug Administration (FDA)-approved medical countermeasures for radiation induced macrophage dysfunction. Herein, we examined cell proliferation and MAPK signaling in macrophage cell lines derived from male and female mice after ionizing radiation (IR) and compared gender radiosensitivity. ***Materials and Methods***: RAW macrophages from male BALB/C mice and J774 macrophages from female BALB/C mice were propagated following ATCC's instructions. Cells grew to 70-95% confluence in culture flasks and were then seeded in either 96-well plates for proliferation analysis or 6-well plates for immunoblotting analysis and cytokine/chemokine measurements. Cells were exposed to 60Co radiation at LD50/2 with a dose rate of 0.4 Gy/min. Results: The average diameter of RAW cells and J774 cells were 21 µm and 16 µm, and their untreated doubling times were 18.5 h and 15.4 h, respectively. IR reduced cell proliferation in RAW cells mediated by AKT, ERK, JNK and Wnt pathways and in J774 cells mediated by JNK and Wnt pathways, as determined using specific inhibitors of AKT, MAPK, mTOR, or Wnt. Dual-specific phosphatases (DUSPs) deactivate MAPKs. In RAW cells, IR increased DUSP4 and decreased ERK phosphorylation. In contrast, in J774 cells, IR decreased DUSP10 and increased p38 phosphorylation. When cells were treated with BCI (a DUSP inhibitor) at 1-5 µM 1 h prior to IR, BCI fully protected RAW cells from IR and decreased ERK phosphorylation, whereas BCI only partially protected J774 cells from RI, increased AKT phosphorylation, and decreased p38 phosphorylation. BCI inhibited IL-6, IL-9, IL-13 and IFN-γ expression in RAW cells while it increased IL-1β, IL-4, IL-10, MIP-1, and IL-18 expression in J774 cells. However, BCI inhibited the IR-induced miR-34a (a promoter of apoptotic cell death and cell cycle arrest) in both cell types. ***Conclusion:*** The results indicate that BCI may be a novel prophylactic protectant against RI in male and female macrophages and male macrophages may be more radiation-sensitive than female ones.

INTRODUCTION

Radiation injury (RI) occurs and manifests principally as hemopoietic acute radiation syndrome (ARS), GI ARS and/or cutaneous ARS. Macrophages are present in every organ and tissue, perform important host defense functions to physical injury, and are essential to innate immunity, but how radiation effects on their function are understudied. There are no U.S. Food and Drug Administration (FDA)-approved medical countermeasures for radiation induced macrophage dysfunction. Herein, we examined cell proliferation and MAPK signaling in macrophage cell lines derived from male and female mice after ionizing radiation (IR) and compared gender radiosensitivity.

METHODS

Cells: RAW macrophages from male BALB/C mice and J774 macrophages from female BALB/C mice were propagated following ATCC's instructions.

Irradiation: Cells were exposed to ^{60}Co radiation at LD50/2 with a dose rate of 0.4 Gy/min.

Cell Proliferation: CellTier 96 AQueous One Solution Assay at 490 nm on 24 h and 48 h.

Western Blot: Dual-Specificity Phosphatase (DUSP), AKT, ERK, JNK, p38 and their phosphorylation were detected.

Cytokine/Chemokine profiling: 32-plex and 9-plex kits were used with Luminex to measure cytokines and chemokine on day 1 and day 2 after IR.

miR-34a measurement: A promoter of apoptotic cell death and cell cycle arrest) in both cell types. total RNA (including small RNAs) were extracted from frozen tissues using mirVana miRNA Isolation Kits . Reverse transcription (RT) was performed, and the resulting cDNAs were quantitatively amplified for miR-34a and U6.

RESULTS

Fig. 1. Dose-Response Curves of Macrophages on 24 h and 48 h Post-IR

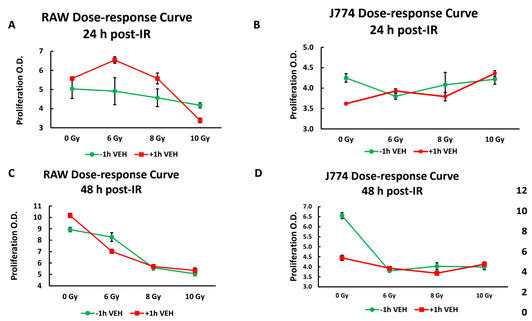


Fig. 1. Dose-Response Relationship in RAW and J774 cells on 24 and 48 h after IR

Fig. 2. Representative Images Photographed on 48 h After IR

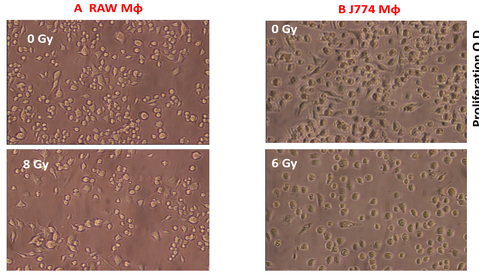


Fig. 2. Representative images taken on 48 h after IR.

Table 1. AKT, MAPK, and Wnt involvement detected after IR. The IR-induced proliferation decrease was blocked by AKT, ERK, JNK, p38 and Wnt in RAW cells and by JNK, p38, and Wnt in J774 cells. (Results from Code 20241001)

	RAW cells	RAW cells	J774 cells	J774 cells
	24 h	48 h	24 h	48 h
Radiation dose	8 Gy	8 Gy	6 Gy	6 Gy
AKTi	↓	↑	↓	↓
MEKi	↓	↓	↓	↓
ERKi	↓	↑	↓	↓
JNKi	↑	↑	↑	↑
P38i	↑	↓	↑	↓
EVEi	↓	↓	↓	↓
RAPAi	↓	↑	↓	↑

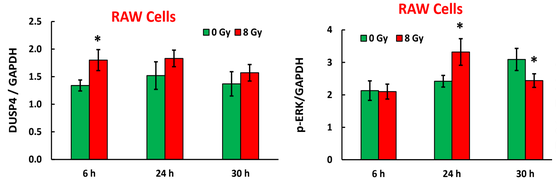


Fig. 3. IR increases DUSP4 and decreased p-ERK in RAW cells. *p<0.05 vs. 0 Gy.

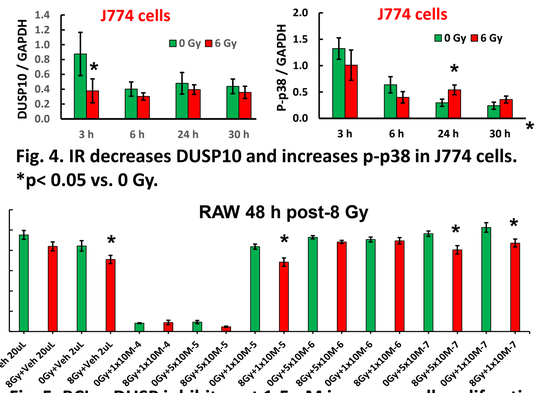


Fig. 4. IR decreases DUSP10 and increases p-p38 in J774 cells. *p< 0.05 vs. 0 Gy.

Fig. 5. BCI, a DUSP inhibitor, at 1-5 µM increases cell proliferation in RAW cells by hyperactivating ERK after IR. p< 0.05 vs. 0 Gy.

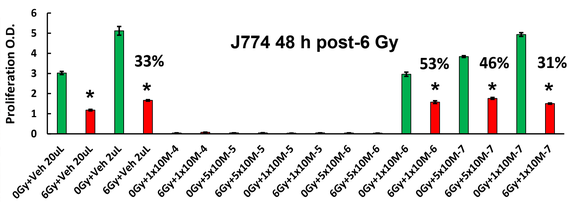
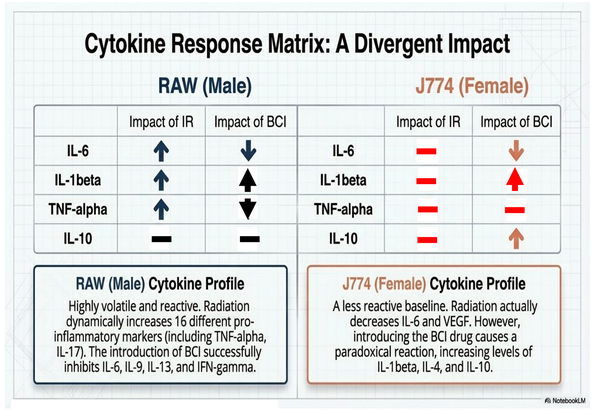


Fig. 6. BCI, a DUSP inhibitor, at 0.5-1 µM increases cell proliferation in J774 cells by hyperactivating AKT and hypoactivating p38 after IR. p< 0.05 vs. 0 Gy.



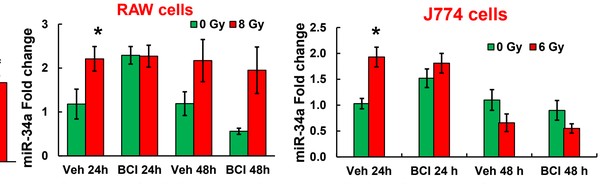
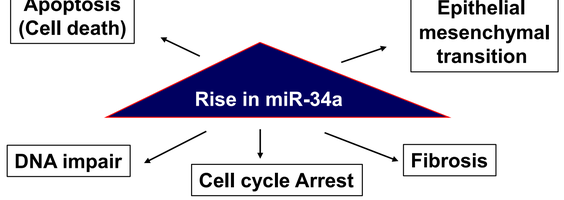


Fig. 7. BCI inhibits the IR-induced miR-34a increases in both RAW and J774 cells. *p<0.05 vs. 0 Gy.

SUMMARY/DISCUSSION

1. Macrophages originate rom monocytes and are present in every organ and tissue.
2. M1: proinflammatory activity (IL-1, IL-6, IL-12, IL-23, TNF-α); tissue damage.
   M2: anti-inflammatory activity (IL-10, TGF-β, VEGF); tissue repair/regeneration
3. IR increased DUSP4 and decreased p-ERK in RAW Mϕ. IR decreased DUSP10 and increased P-p38 in J774 Mϕ.
4. BCI (a DUSP inhibitor) fully protected RAW Mϕ proliferation from IR by ERK hyperactivation, whereas BCI partially protected J774 Mϕ proliferation from IR due to JNK hyperactivation even though AKT was hyperactivated and p38 was hypoactivated.
5. In RAW Mϕ, IR dynamically increased IL-1, IL-3, IL-5, IL-12p40, IL-13, IL-17, Eotaxin, G-CSF, IFN-γ, Rantes, TNF-α, LIF, M-CSF, MIG, MIP-2, and PDGF-bb, and BCI inhibited only the IR-induced increases in IL-6, IL-9, IL-13, TNF-α, and IFN-γ.
6. In J774 Mϕ, IR decreased IL-6, M-CSF, MIP-2, and VEGF but increased MIP-1β, LIF, and MIG, and BCI increased the IR-induced increases in IL-1β, IL-4, IL-10, MIP-1, and IL-18.
7. IR increased miR-34a in both RAW and J774 Mϕ, BCI inhibited this increase.

Effects of IR-Induced Increases in MiR-34a That Is Inhibited by BCI Pretreatment



CONCLUSIONS

Our data suggests that the DUSP inhibitor BCI can protect male cells by hyperactivating specific survival pathways, though its efficacy varies significantly by sex.

The findings indicate that male Mϕ are more sensitive to radiation-induced changes than female ones, aligning with broader observations of higher radio-resistance in females.

BCI may be a prophylactic protectant for male and female macrophages.

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