Delayed Effects of Acute Radiation Exposure on the Cardiovascular System using a Murine Model of the Hematopoietic Acute Radiation Syndrome

Beatrice Thungu[1], Miguel Ortiz[1], Joseph L. Unthank[1,2], Christie M. Orschell[3], Steven J. Miller[1,2]

[1]Department of Surgery, [2]Cellular and Integrative Physiology, and [3]Department of Medicine Indiana University School of Medicine, Indianapolis, IN

INDIANA UNIVERSITY SCHOOL OF MEDICINE

DEPARTMENT OF SURGERY INDIANA UNIVERSITY School of Medicine

ABSTRACT

Introduction. Exposure to high level radiation from accidents or belligerent activities results in acute and chronic organ damage. The hematopoietic system is the most sensitive organ to radiation damage (2-10 Gy) and results in the hematopoietic acute radiation syndrome (H-ARS). Survivors of H-ARS are plagued months to years later with delayed effects of acute radiation exposure (DEARE), characterized by chronic illnesses affecting multiple organ systems. Previous results using the murine H-ARS model showed numerous kidney and heart DEARE-related pathologies similar to humans, including tissue fibrosis and elevated blood urea nitrogen. The goal of this study was to utilize the murine H-ARS model to determine possible roles for abnormal iron metabolism, inflammation, oxidant stress, and senescence in the development of cardiac DEARE.
Methods. Mice (C57BL/6; 12 week-old) received total body irradiation (TBI: ~8.5-8.7 Gy, ^{137}Cs, $LD_{50}$to LD_{70}) and hearts were harvested at various times post-TBI from H-ARS survivors. Paraffin tissue sections were stained with hematoxylin/eosin or Perls Prussian Blue, or reacted with a macrophage –specific antibodies (F4/80). Total RNA was purified from fresh tissue and changes in mRNA expression were assessed by real-time PCR for the senescence marker p16 and NADPH oxidase subunits Nox2, Nox4, or p47phox.
Results/Significance. Compared to age-matched non-irradiated controls (NI), tissue iron deposits were increased in irradiated (IR) hearts at 4 months, and progressively declined with time post-TBI. Numbers of macrophages were greater in IR vs. NI sections at all time points and decreased with time post-TBI. Nox2 and Nox4 mRNA expression was increased at both 9 and 21 months post-TBI, but p47phox increased only at 21 months. Expression of p16 in IR heart was increased at 7, but not at 22 months post-TBI. Taken together, the results indicate abnormal iron metabolism, inflammation, oxidant stress, and early senescence may contribute to development of cardiac DEARE.

INTRODUCTION

- High levels of radiation exposure from accidents or belligerent activities like terrorism results in acute and chronic organ damage.
- Exposure of 2 – 10 Gy in mice results in the hematopoietic acute radiation syndrome (H-ARS), which if untreated results in death within weeks (Plett et al., Health Phys 103:343, 2012).
- Survivors of H-ARS are plagued months to years later with delayed effects of acute radiation exposure (DEARE), characterized by chronic illnesses affecting multiple organ system like the cardiovascular system.
- Previous data (Unthank et al. Health Phys, 109:511-21, 2015) using the murine H-ARS model showed numerous heart DEARE-related pathologies similar to humans, including tissue fibrosis and pericardial thickening (Figure 1).
- In order to investigate mechanisms related to cardiac DEARE, this study examined the possible role of abnormal iron metabolism, inflammation, oxidative stress, and senescence post–total body irradiation (TBI).

Cardiac DEARE Fibrosis & Pericardial Thickening

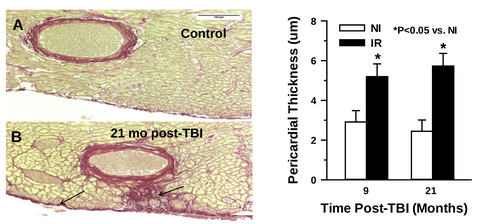


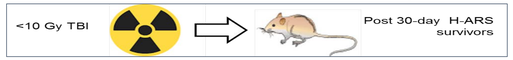
Figure 1. Images of ventricular cross-sections of an age-matched non-irradiated control (A) and 21 mo post-TBI stained with picrosirius red (B). Increased peri-arterial and pericardial staining for fibrosis was consistent in irradiated mice. The plot shows the calculated average thickness of the pericardial collagen which significantly increased with time post-TBI in irradiated (IR) vs. non-irradiated (NI).

OBJECTIVE

To assess possible roles for abnormal iron metabolism, inflammation, oxidant stress, and senescence in the development of cardiac DEARE.

METHODS

Outline of Cardiac-DEARE study



DEARE Related Tissue Pathology/Damage

Heart Analyses: 4-22 month post-total body irradiation (TBI)

- Assessment of Abnormal Iron Metabolism (Perls Prussian Blue)
- Determination of Inflammation (Macrophages).
- Determination NADPH oxidase subunit expression (Nox2, Nox4, and p47phox).
- Determination of senescence (p16 expression).

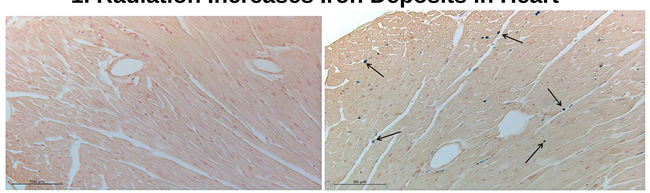
Animals and tissue collection. All procedures were approved by the Indiana University School of Medicine IACUC. Mice (male and female C57BL/6) received total body irradiation (TBI) of 8.5-8.7 Gy (LD50 to LD70) with ^{137}Cs at 12 weeks-of-age. Heart tissue was harvested at various points post-TBI from H-ARS survivors paired with age-matched non-irradiated controls.

Histology/Immunohistochemistry. Tissues were fixed in neutral buffered formalin and paraffin-embedded. Tissue sections were stained with hematoxylin/eosin or Perls Prussian Blue, or reacted with a macrophage–specific antibody (F4/80).

Real-time Quantitative PCR. Total RNA was purified, reverse transcribed, and changes in mRNA expression were assessed by real-time PCR for the senescence marker p16 and NADPH oxidase subunits Nox2, Nox4, or p47phox as an indicator of oxidant stress.

RESULTS

1. Radiation Increases Iron Deposits in Heart



Non-Irradiated Irradiated

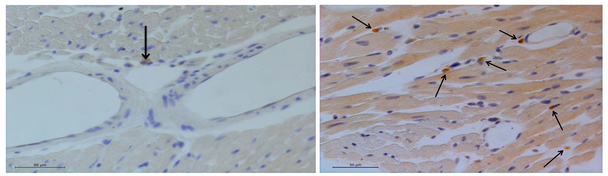
Figure 2. The presence of myocardial and perivascular iron deposits were assessed in irradiated and age-matched non-irradiated heart tissue sections with Perls Prussian Blue staining. Representative images from 4 months post-TBI are shown, and arrows indicate areas of iron deposition.

2. Quantitation of Cardiac DEARE-related Iron deposits

Time Post-TBI	NI	IR
4 mo	0.30	10.41
6 mo	0.27	7.78
13 mo	0.0	1.83
18 mo	ND	1.77

Table 1. Quantitation of heart iron deposits. The table shows the average number of distinct nodes of Perls staining per high power field in non-irradiated (NI) and irradiated (IR) tissues over time. Note that the average number decreases with time post-TBI.

3. Cardiac DEARE-related Inflammation (Macrophages)



Non-Irradiated Irradiated

Figure 3. The presence of macrophages was assessed in irradiated and age-matched non-irradiated heart tissue sections using a macrophage – specific antibody (F4/80). Representative images from 4 months post-TBI are shown, and arrows indicate presence of macrophages. Macrophage numbers appeared to be increased in irradiated tissue.

4. Quantitation of Cardiac DEARE-related Macrophage

Time Post-TBI	NI	IR
4 mo	16.3	101.3
18 mo	9.5	21.6

Table 2. The average number of macrophages per high-power field were higher at 4 and 18 month in irradiated (IR) heart tissue compared to the non-irradiated (NI). Overall, the average number of macrophages decreased with time post-TBI.

5. Cardiac DEARE-related oxidant stress

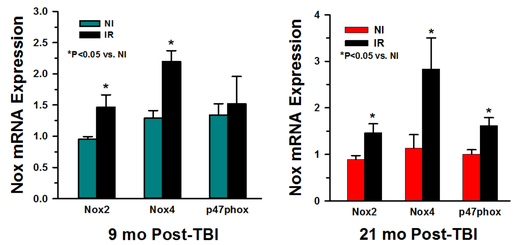


Figure 4. Real-time PCR was used to assess changes in heart NADPH oxidase (Nox) subunit mRNA expression at 9 and 21 mo post-TBI as an indicator of oxidant stress. Nox2 and Nox4 were significantly increased at both 9 and 21 mo, but p47phox was increased only at 21 mo (n=5-6).

6. Cardiac DEARE-related Senescence

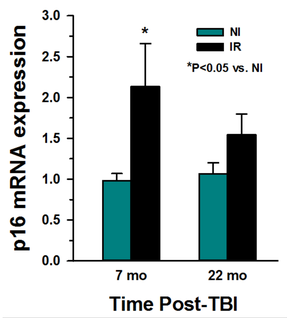


Figure 5. Real-time quantitative PCR was used to access changes in p16 mRNA expression in irradiated (IR) vs. non-irradiated (NI) heart, which was significantly increased at 7 but not 22 months post-TBI (n=5-6).

SUMMARY/CONCLUSIONS

- **Iron Deposits:** Increased iron deposits (likely hemosiderin) in IR tissue at 4 months and a continual decline with time post-TBI (Fig. 2 & Table1) indicates abnormal iron metabolism may function as an early event in development of cardiac DEARE pathology and dysfunction.
- **Macrophages:** Increased macrophage numbers at 4 and 18 months in IR vs. NI tissue and an overall decrease with time post-TBI (Fig. 3, Table 2) suggests inflammation may be an early contributor to development of cardiac DEARE pathology and dysfunction, and may be correlated with the presence of iron.
- **Senescence:** Elevated p16 expression at 7 but not 22 months in IR vs. NI tissue (Fig. 5) suggests cellular senescence may be an early event contributing to accelerated aging, oxidant stress, and DEARE-related pathology and dysfunction.
- **Oxidant stress:** The pattern of increased Nox subunit expression at 9 and 21 months (Fig. 4) indicates a potential role for oxidant stress in the progression of cardiac DEARE pathology and dysfunction.
- Taken together, the results indicate abnormal iron metabolism, inflammation, and early senescence occur prior to development of cardiac fibrosis (Fig. 6) and represent potential targets, along with oxidant stress, for mitigation of DEARE-related cardiac pathology and dysfunction.

Comparison of Early and Late Cardiac DEARE

Cardiac DEARE Progression

Early Events (4-7 months)
- Iron Deposits ↑
- Inflammation (Macrophages) ↑
- Senescence ↑
- Oxidant Stress??

Late Events (9-22 months)
- Iron Deposits ↓
- Inflammation (Macrophages) ↓
- Senescence ↓
- Oxidant Stress ↑
- Fibrosis/Pericardial Thickening

Figure. 6. Early effects of cardiac DEARE in irradiated tissue show increased iron, inflammation (macrophages) and senescence. In contrast, late effects show decreases with the exception of oxidant stress that increased. Tissue fibrosis/pericardial thickening was also observed as a late occurrence (Fig. 1).

FUTURE WORK

- Determine if oxidative stress occurs at early time points.
- Correlate changes in protein markers of oxidant stress such as nitrotyrosine and 8-hydroxy-2-deoxy Guanosine with Nox subunit expression.
- Determine if the inflammation marker ICAM-1 correlates with the presence of macrophages in DEARE.
- Develop mitigators of DEARE organ pathology and dysfunction.

ACKNOWLEDGEMENTS

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