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

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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 systems 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

RESULTS

1. Radiation Increases Iron Deposits in Heart

Cardiac DEARE Fibrosis & Pericardial Thickening

Figure 1. Images of ventricular cross-sections of an age-matched non-irradiated (A) and 21 mo post-TBI (B) hearts stained with Perls Prussian blue. Increased periarterial and pericardial staining for iron 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).

2. Quantitation of Cardiac DEARE-related Iron deposits

<table>
<thead>
<tr>
<th>Time Post-TBI</th>
<th>Non-Irradiated</th>
<th>Irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mo</td>
<td>0.30</td>
<td>10.41</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.25</td>
<td>7.78</td>
</tr>
<tr>
<td>13 mo</td>
<td>0.0</td>
<td>1.83</td>
</tr>
<tr>
<td>18 mo</td>
<td>ND</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Table 1. Quantification 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.

METHODS

Outline of Cardiac-DEARE study

- HEART ANALYSES: 4-22 month post-total body irradiation (TBI)
- DEARE Related Tissue Pathology/Damage
- HEART ANALYSES: 4-22 month post-total body irradiation (TBI)

- Determination NADPH oxidase (Nox) subunit mRNA expression at 9 and 21 mo post-TBI as an indicator of oxidant stress.
- Determination of Inflammation (Macrophages).
- Determination of Senescence (p16 expression).

- HEART ANALYSES: 4-22 month post-total body irradiation (TBI)

- Assessment of Abnormal Iron Metabolism (Perls Prussian Blue).
- Determination of inflammation (macrophages).
- Determination of NADPH oxidase subunit expression (Nox2, Nox4, and p47phox).

- HEART ANALYSES: 4-22 month post-total body irradiation (TBI)

- Determination of oxidant stress (Nox2, Nox4, and p47phox).
- Determination of inflammation (macrophages).
- Determination of senescence (p16 expression).

ANIMALS AND TISSUE COLLECTION.

All procedures were approved by the Indiana University School of Medicine’s Animal Care Committee. Mice (male and female C57BL/6) received total body irradiation (TBI) of 8.5-8.7 Gy (137Cs) with or without subsequent 9 Gy TBI (2 weeks post-TBI). Arteries were harvested at various times post-TBI from H-ARS survivors and age-matched non-irradiated (NI) controls.

HISTOLOGY/IMMUNOHISTOCHEMISTRY.

Tissues were fixed in neutral buffered formalin and sections were stained with hematoxylin/eosin or Prussian blue, or reacted with a macrophage-specific antibody (F4/80).

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SUMMARY/CONCLUSIONS

Iron Deposits: Increased iron deposits (likely hemosiderin) in IR tissue at 4 months and a continual decline with time post-TBI (Figure 2) indicates normal 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 (Figure 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 (Figure 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 (Figure 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 (Figure 6) and represent potential targets, along with oxidant stress, for mitigation of DEARE-related cardiac pathology.

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-deoxyguanosine 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.

FUTURE WORK

Determine if oxidative stress occurs at early time points.

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