Delayed effects of acute radiation exposure (DEARE) in a murine model of the hematopoietic acute radiation syndrome: Multiple-organ injury consequent to total body irradiation

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ABSTRACT

Introduction. Victims of radiation exposure from terrorist activity, radiation accidents or radiologic warfare will face a variety of acute and chronic organ injuries requiring multi-faceted approaches to treatment. The hematopoietic system is the most sensitive tissue to radiation damage, resulting in the hematopoietic acute radiation syndrome (H-ARS) after exposures of 2-10 Gy in mice. If untreated, H-ARS results in death within weeks from opportunistic infection and/or hemorrhage due to loss of neutrophils and platelets, respectively. However, survivors of ARS are plagued months to years later in life by delayed effects of acute radiation exposure (DEARE), a myriad of chronic illnesses affecting multiple organ systems believed to be due to persistent systemic oxidative stress, inflammation, fibrosis and loss of stem cell self-renewal. Fibrosis and collagen deposition disrupt both normal tissue structure and function and are common to organs with late radiation injury including the kidney and heart after radiation doses ≥15Gy, but have not been shown to exist after doses as low as those used in the H-ARS model (8Gy). The goal of this study was to determine the extent, if any, of heart and kidney DEARE in survivors of H-ARS.
Methods. Mice (male and female C57BL/6) received total body irradiation (TBI; $LD_{50/30}$ to $LD_{70/30}$) and kidney and heart were harvested at 9 and 21 months from the H-ARS survivor mice. Tissues were fixed in neutral buffered formalin, paraffin embedded and sectioned, then stained with hematoxylin/eosin (H&E), trichrome, or picosirius red. Serum was collected at 4.3, 9, and 21 months post-TBI and analyzed for blood urea nitrogen (BUN) as an indicator of kidney function. Total RNA was purified from heart and relative changes in NADPH oxidase 2 (Nox2) mRNA expression were assessed by quantitative real-time PCR.
Results/Significance. Compared to age-matched non-irradiated controls (NI), renal pathology at 9 months post-TBI was manifest primarily as enlargement of Bowman's capsule and glomerosclerosis along with limited interstitial fibrosis. By 21 months there was progression of these pathologies as well as extensive interstitial fibrosis, tubular atrophy, cysts, and atubular glomeruli, all of which were more pronounced in TBI mice compared to NI. Consistent with the renal pathology, BUN in TBI mice was significantly increased at 9 and 21 months post-TBI vs. 4.3 months, but normal in NI mice at all time points. In the heart, pericardial, perivascular and interstitial fibrosis were observed at 9 months with increased severity at 21 months post-TBI compared to NI. The perivascular fibrosis was associated with increased medial layer collagen and apparent loss of vascular smooth muscle cells. Nox2 mRNA in heart was increased at 9 and 21 months post-TBI, indicating an increase in oxidant stress. To our knowledge, such striking heart and kidney damage has not been documented after radiation doses as low as those in our H-ARS model (~8Gy) and indicate that DEARE is a concern for individuals exposed to radiation doses previously thought to not elicit late effects.

BACKGROUND

- Acute, high dose radiation exposure, such as that resulting from terrorist use of radiation or radiation accident, results in acute and chronic organ injury requiring multiple approaches for treatment.
- Exposures of 2-10 Gy in mice result in the hematopoietic acute radiation syndrome (H-ARS), which if untreated results in death within weeks.
- Survivors of H-ARS are plagued months to years later in life by delayed effects of acute radiation exposure (DEARE).
- DEARE results in multiple chronic illnesses affecting multiple organ systems characterized by systemic oxidative stress, inflammation, and tissue fibrosis, which affects tissue structure and function.
- DEARE-related fibrosis and collagen deposition have been shown in heart and kidney at organ-specific doses ≥15Gy, but this pathology has not been shown to occur following the lower doses used in the H-ARS total body irradiation mouse model.
- This study utilized the H-ARS mouse model to determine the extent to which, if any, DEARE-related pathology occurs at the lower radiation doses used for this model.

OBJECTIVE

Determine the extent to which DEARE-related heart and kidney pathology and dysfunction occur in a mouse model of H-ARS survivors.

MATERIALS and METHODS

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; LD50/30 to LD70/30) with ^{137}Cs at 12 weeks-of-age. After 30 days, irradiated survivors were paired with age-matched non-irradiated controls, and kidney and heart were harvested at 9 and 21 months (n=4-5 per group). Serum was collected at 4, 9, and 21 mo-TBI and from non-irradiated control mice.

Histology and scoring. Tissues were fixed in neutral buffered formalin, paraffin embedded and sectioned, then stained with hematoxylin/eosin (H&E), Masson's trichrome, or picrosirius red. Digital images of tissue sections were acquired and evaluated for fibrosis and other specific pathological characteristics using image analysis software (Image J) when appropriate. Renal histology scores were derived by grading five kidney pathology characteristics (Scoring Table) for individual mice within each group. The mean group score for each characteristic was calculated, and group characteristic scores were added to obtain a total histology score (Table 1).

Histological Scoring Table

Abnormality	Score
% of Sclerosed glomeruli (20 glomeruli/slide)	
0-5%	0
6-25%	1
26-50%	2
51-75%	3
> 75%	4
Hypertrophied glomeruli (diameter>120um)	
none	0
>1	1
Tubularized glomeruli (# present/slide)	
none	0
>1	1
Interstitial fibrosis	
Normal collagen	0
Diffuse, minimal	1
Diffuse, minimal with focal	2
Diffuse, significant	3
Protein Casts (# per slide)	
0-30	0
31-60	1
>60	2

Blood urea nitrogen (BUN). BUN in serum was determined using a Pointe Scientific 180 Quick Touch analyzer and reagents with a 20 mg/dl standard. All samples were stored at -80 ºC prior to assay. Evaluation of potential effects on BUN due to blood collection methods, gender, or radiation dose indicated no statistical difference in the values obtained at any time post-TBI.

Real-time Quantitative PCR. Total RNA was purified from heart and relative changes in NADPH oxidase 2 (Nox2) mRNA expression were assessed by quantitative real-time PCR.

RESULTS

Renal Interstitial Fibrosis Increased with Time After Total Body Irradiation (TBI)

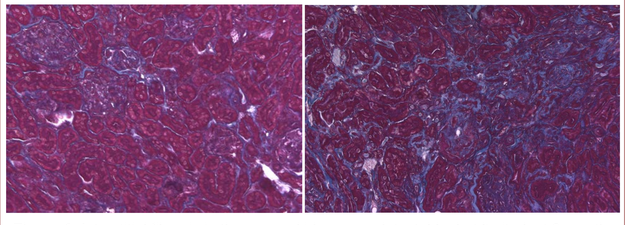

Figure 1. Interstitial fibrosis in the mouse kidney was detectable, but limited, at 9 months post-TBI (left). At 21 months post-TBI (right) interstitial fibrosis had become extensive. Thus, a clear progression of pathology is evident between time points in the irradiated mouse kidney.

Abnormal Glomeruli Correlated with DEARE

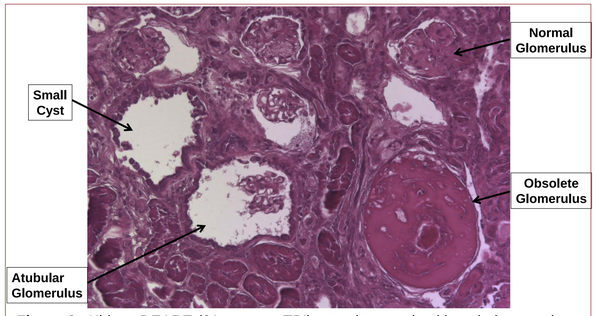

Figure 2. Kidney DEARE (21 mo post-TBI) was characterized by tubular atrophy present in the interstitium due to loss of tubules. The atubular glomerulus also showed enlargement of Bowmen's Capsule with apparent tubularization of epithelial cells. An obsolete glomerulus contains a retracted tuft and widespread collagenous material.

Glomerular Sclerosis Developed with DEARE

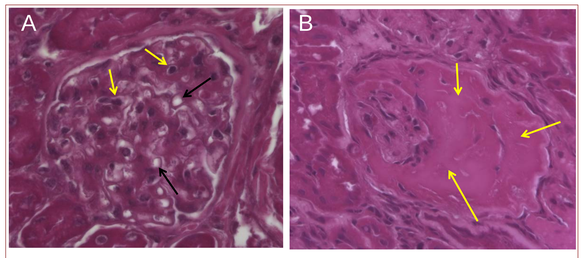

Figure 3. A. Normal glomeruli showing evenly dispersed cellular material with several nuclei (yellow arrows) and capillaries (black arrows). B. Sclerosed glomeruli (21 mo post-TBI) with lack of cellular material replaced with collagen (arrows) and significantly decreased nuclei and capillaries.

Renal DEARE Histological Scoring

	9 mo NI	9 mo IR	21 mo NI	21 mo IR
Sclerosed glomeruli 0-4	0	1.75	0	3
Hypertropied glomeruli 0-1	0	0	0	1
Tubularized glomeruli 0-1	0	0	0	0.75
Interstitial fibrosis 0-3	0	1.5	1.25	3
Protein casts 0-2	0.2	0.25	0.75	2
TOTAL Score	**0.2**	**3.5**	**2**	**9.75**

Table 1. Renal pathology increased with time post-TBI (IR) compared to non-irradiated controls (NI). Renal pathology mean scores were calculated for each mouse group characteristic and then added to generate a total group score.

Renal Function Decreased with Time Post-TBI

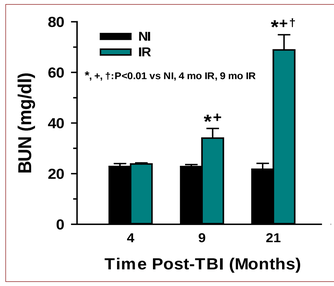

Figure 4. Blood urea nitrogen (BUN) was determined as an indicator of kidney function. BUN was significantly elevated in irradiated (IR) mice at 9 and 21 months compared to 4 months, indicating a decrease in function with time. BUN was unchanged with aging in non-irradiated (NI) mice.

Cardiac DEARE Pathology

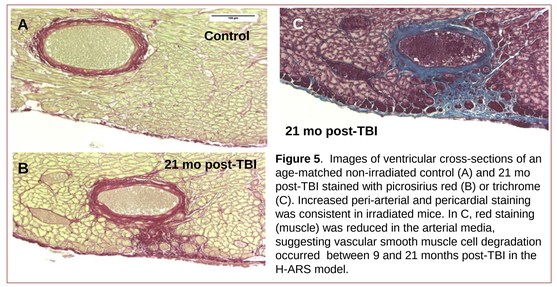

Figure 5. Images of ventricular cross-sections of an age-matched non-irradiated control (A) and 21 mo post-TBI stained with picrosirius red (B) or trichrome (C). Increased peri-arterial and pericardial staining was consistent in irradiated mice. In C, red staining (muscle) was reduced in the arterial media, suggesting vascular smooth muscle cell degradation occurred between 9 and 21 months post-TBI in the H-ARS model.

Pericardial Collagen and Cardiac Oxidant Stress Increased with DEARE

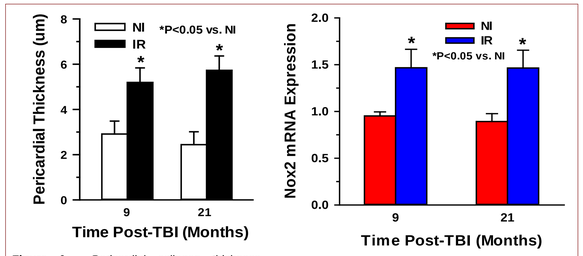

Figure 6. Pericardial collagen thickness significantly increased with time post-TBI in irradiated (IR) vs. non-irradiated (NI) heart. The plot shows the calculated average thickness of the pericardial collagen (see Fig. 5).

Figure 7. NADPH oxidase 2 (Nox2) mRNA expression in the left ventricle was significantly increased in irradiated (IR) mice compared to the non-irradiated controls (NI) at both 9 and 21 mo post-TBI.

SUMMARY/CONCLUSIONS

- Significant DEARE-related renal pathology occurred in H-ARS survivor mice from 9 to 21 months post-TBI (Figs. 1 – 3 and Table 1).
- Decreased renal function, as assessed by BUN, also was associated with DEARE in H-ARS survivor mice (Fig. 4).
- Significant cardiovascular DEARE characterized by fibrosis and collagen deposition occurred in hearts of H-ARS survivors 21 months post-TBI (Figs. 5 & 6).
- Cardiac oxidant stress, indicated by elevated Nox2 expression, was apparent at 9 and 21 months post-TBI in H-ARS survivor mice (Fig. 7).
- Taken together, the results indicate that renal and cardiac DEARE occur at lower radiation doses than previously thought. Thus, the H-ARS mouse model is suitable for study of mechanisms related to DEARE-related pathology, especially in kidney.

Future Work

- Evaluate changes in organ pathology and function at additional time points.
- Determine age-related effects on DEARE development.
- Evaluate radiologic mitigators of H-ARS on DEARE organ pathology and dysfunction.