Acute Total Body Ionizing Radiation Induces Long-Term Cardiac Effects and Immediate Changes in Oxidative Carbonylation of Cardiac Troponin T in the Rat

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Abstract

Radiation-induced heart disease represents a significant health risk in the event of an accidental radiation exposure as well as to cancer patients who receive acute doses of irradiation as part of radiation therapy. We utilized the Wistar-Kyoto and spontaneously hypertensive rat models, previously shown to demonstrate drug-induced cardiomyopathy, to evaluate the acute and long-term effects of sub-lethal total body irradiation at two, four, and fifty-two weeks.

We examined irreversible oxidative protein carbonylation in the heart immediately following irradiation. Both males and females sustained reduced growth and anemic conditions over a one-year period as reflected by reduced body weight and low red blood cell count. Increased inflammation was detected via elevated IL-6 serum levels selectively in males at four weeks. Serum cardiac troponin T and I analyses revealed signs of cardiomyopathy at earlier timepoints, but high variability was observed, especially at one year.

Echocardiography at two weeks following 5.0Gy treatment revealed inflammation and a decrease in cardiac output in the short term, but significant differences were not observed at the one-year timepoint. Cardiac output was decreased in both males and females after two weeks, and statistically significant in females. Following 10.0Gy irradiation, the heart tissue showed an increase in total protein oxidative carbonylation accompanied by DNA damage indicated γ-H2AX.

Using proteomic analyses, several novel proteins showed a marked carbonylation profile including those associated with mitochondrial homeostasis and cardiac contractility. Overall, we present findings of acute oxidative protein, DNA damage, and long-term cardiomyopathy in the irradiated animals, along with oxidative proteomic markers of acute damage.

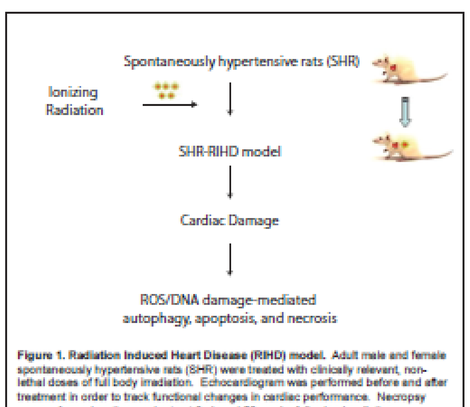

Figure 1. Radiation Induced Heart Disease (RIHD) model. Adult male and female spontaneously hypertensive rats (SHR) were treated with clinically relevant, non-lethal doses of full body irradiation. Echocardiogram was performed before and after treatment in order to track functional changes in cardiac performance. Necropsy was performed on three cohorts at 2, 4, and 52 weeks following irradiation.

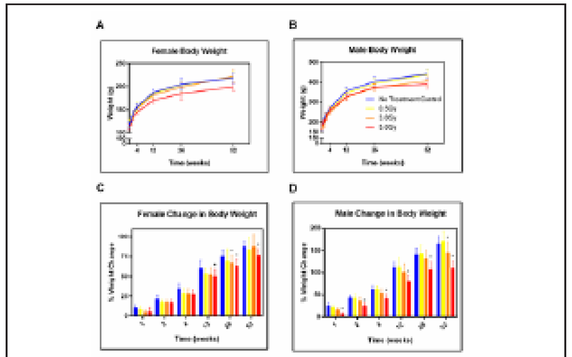

Fig 1. Total body irradiation negatively impacts growth for up to one year. The average female (A) and male (B) body weight is displayed over one year. The average change in female (C) and male (D) body weight is displayed over one year. From left to right, groups of bars denote no treatment control, 0.5Gy, 3.0Gy, and 5.0Gy for each timepoint. Error bars = SD; NT=no treatment; *p<0.05 wrt NT, n=10

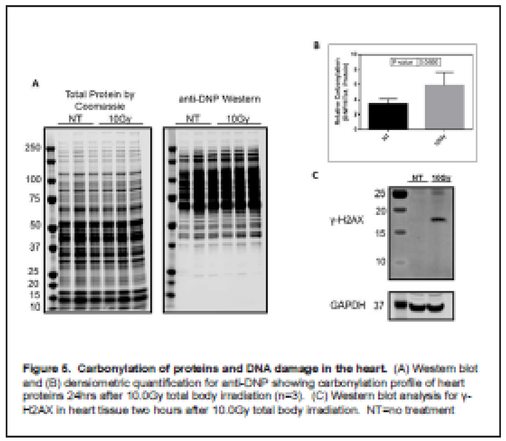

Figure 5. Carbonylation of proteins and DNA damage in the heart. (A) Western blot and (B) densiometric quantification for anti-DNP showing carbonylation profile of heart proteins 24hrs after 10.0Gy total body irradiation (n=3). (C) Western blot analysis for γ-H2AX in heart tissue two hours after 10.0Gy total body irradiation. NT=no treatment

Spot ID	Carbonyl Fold Change	Identity	Known Function
1	3.6	Serum albumin	Maintain oncotic pressure, carries proteins
2	5.9	Serotransferrin	Iron binding, transport
3	<0.8	60 kDa heat shock protein	Maintain imported protein folding
4	3.5	Serum albumin	Maintain oncotic pressure, carries proteins
5	5.9	ATP synthase subunit alpha	ATP synthesis and ion transport
6	5.7	ATP synthase subunit alpha	ATP synthesis and ion transport
7	2.8	ATP synthase subunit beta	ATP synthesis and ion transport
8	2.0	Alpha-enolase	Enzyme; glycolysis, glycolysis, growth control, hypoxia tolerance and allergic response
9	5.3	Serum albumin	Maintain oncotic pressure, carries proteins
10	3.6	Troponin T, cardiac	Cardiac structural protein
11	3.6	Isocitrate dehydrogenase [NAD] subunit alpha	Catalyzes the decarboxylation of isocitrate
12	5.7	Electron transfer flavoprotein subunit alpha	Accepts electrons from several mitochondrial dehydrogenases
13	-0.6	Heat shock protein beta-6	Cardiac myocyte contractility
14	3.1	Heat shock protein beta-5	Molecular chaperone, HSP

Figure 7. Selected proteins from 2D-PAGE gel of male rat myocardium exhibiting change in carbonylation pattern following irradiation. Fourteen spots were selected for MS analysis, and 11 unique proteins were identified as showing a change in carbonylation level after 24hrs following 10.0Gy irradiation. n=3

		Units	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT
			10^3/uL	10^6/uL	g/dL	%	fL	pg	g/dL	%	10^3/uL
Male	NT	AVG	8.8	9.28	14.2	37.8	38.8	15.3	38.3	23.5	607
		SD	1.2	0.56	0.8	1.8	0.5	0.4	0.7	0.9	33
	0.5 Gy	AVG	7.1	9.01	14.0	36.5	40.6	15.6	38.4	23.6	575
		SD	1.1	0.27	0.7	1.2	1.7	1.1	0.9	1.0	51
	3.0 Gy	AVG	6.3	8.52*	13.2*	35.4	42.0*	15.6	37.2*	21.5*	681
		SD	2.2	0.75	0.6	1.4	2.6	0.8	0.5	1.3	58
	5.0 Gy	AVG	5.6*	7.68*	12.2*	33.2*	43.4*	15.8*	36.8*	20.3*	692
		SD	1.4	0.62	0.8	1.8	0.9	0.4	0.4	1.1	134
Female	NT	AVG	5.8	8.2	13.7	36.2	43.9	16.6	37.8	19.9	644
		SD	1.8	0.4	0.6	1.2	0.9	0.4	0.6	0.6	58
	0.5 Gy	AVG	4.7	8.0	13.2	36.3	44.8	16.7	37.4	19.8	678
		SD	1.2	0.4	1.2	1.8	2.8	0.5	1.8	0.8	152.7
	3.0 Gy	AVG	5.5	8.19	13.5	36.5	44.7	16.5	37.8*	19.7	585*
		SD	1.2	0.59	0.7	1.5	1.0	0.3	0.5	1.0	58
	5.0 Gy	AVG	5.4	7.62*	12.6*	34.4*	45.1*	16.5	36.6*	19.8*	678
		SD	1.8	0.56	0.6	1.3	0.5	0.3	0.5	0.5	101

Figure 3. Ionizing radiation produces anemic conditions. Complete blood count for female and male rats at 52 weeks after exposure to ionizing radiation. n=10

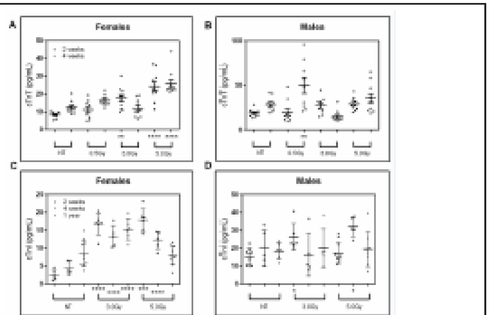

Figure 4. Serum levels of cTnT and cTnI reveal acute cardiomyopathy. Serum cTnT were detected in (A) female and (B) male at 2 and 4 weeks after IR. Serum cTnIcT at necropsy for (A) female and (B) male at 2, 4, and 52 weeks. Error bars = SD; NT=no treatment; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 wrt NT; n=5-10

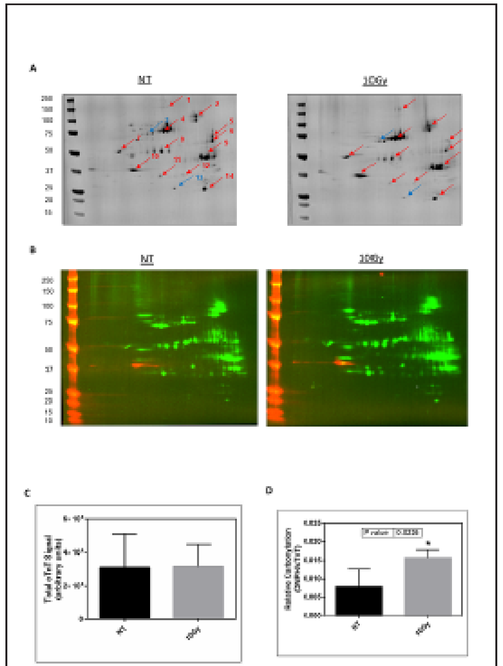

Figure 6. Protein carbonylation in male rat heart at 24hrs. (A) 2D western for carbonylation; increase (red), decrease (blue). (B) Two-color, 2D western showing overlap of cTnT (red) and DNP (green) signals. (C) Densitometry quantification of total cTnT. (D) Densitometry quantification of relative carbonylation of cTnT. Carbonylation signal (DNP) normalized by cTnT. NT=no treatment; n=43

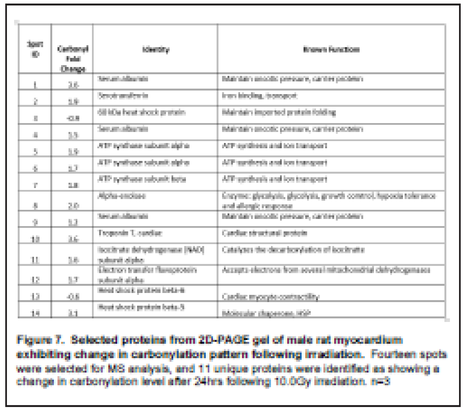

Figure 8. Total body irradiation results in physiological changes to heart. Echocardiography at two weeks documenting left ventricular volume at (A) diastole and (B) systole. (C) Cardiac output in males and females. Bars represent standard deviation. NT=no treatment; n=3; *p<0.05

Highlights

- Cardiac troponin T (cTnT) analysis revealed cardiomyopathy in both 3 and 5Gy groups at early timepoints.
- Fourteen cardiac proteins show a change in carbonylation level following 10Gy irradiation.
- **cTnT, a key protein involved with contractile function,** is susceptible to protein oxidation, specifically carbonylation, following irradiation.

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