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A MOUSE MODEL OF RADIATION-INDUCED CARDIOMYOPATHY

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Keywords

Radiation therapy; cardiomyopathy; heart failure

Radiation therapy (XRT) is an essential component in the management of many types of cancer [1]. XRT of the thorax frequently places the heart in the radiation field, leading to cardiac injury [1–3]. Improvements in early diagnosis and treatment have increased cancer survival, engendering the need to critically evaluate the long-term consequences of XRT [2–3]. From a clinical standpoint, XRT-induced heart injury is heterogeneous. XRT-induced cardiomyopathy may be clinically apparent with an initial early injury or may be latent for many years before presenting with heart failure [1]. From a pathologic standpoint, XRT induced cardiomyopathy is characterized by myocardial fibrosis in absence of left ventricular (LV) dilatation and associated with normal or reduced LV mass [1–3]. Unfortunately, the incidence and pathophysiology of XRT-induced cardiomyopathy remains poorly described due to a lack of prospective data and a latency period that exceeds the follow-up duration of most clinical studies. Given the increasing recognition of XRT-induced heart disease and recent progress in murine cardiac imaging, we performed a systematic study of XRT-induced cardiomyopathy in the mouse.

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Competing interests

The authors declare no competing interests.

Author contributions

EM, PG, DAG and AA designed the study and drafted the manuscript. EM and XD equally contributed to carry out studies. ST and BVT participated in the completion of the studies and analysis of the data. HK carried out the immunohistochemistry experiments. NV and CMB contributed to the final drawing up of the manuscript. All the authors read and approved the final manuscript.

Experiments followed guidelines on the humane use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996) and received local Institutional Animal Care and Use Committee approval. Three-months old C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME) were irradiated with a single 20 Gy dose of XRT (Group I, N=12). Anesthetized mice (pentobarbital 50 mg/kg) were shielded with lead plates leaving the thorax region exposed. XRT was administrated locally with a Trilogy linear accelerator equipped with state of the art guided radiation therapy (IGTR)(Varian, Medical system, Palo Alto, CA). An additional group of mice (Group II, N=6) underwent sham-irradiation. All mice were followed for 6 months (Figure, panel A).

Transthoracic echocardiography was performed under light anesthesia (pentobarbital 50 mg/kg) to determine cardiac dimensions and function using the Vevo770 imaging system (VisualSonics Inc. Toronto, Ontario, Canada) equipped with a 30-MHz probe [4–5]. The thickness of the posterior pericardium and of the mitral valve and aortic valve leaflets was also measured in M-mode, the presence of pericardial fluid was also determined. Finally, left ventricular ejection fraction (LVEF) was measured at rest and 3 minutes after injection with the β-adrenergic receptor agonist isoproterenol (10 ng/mouse, Sigma Aldrich), to measure contractile reserve, defined as percentage change of LVEF within 3 minutes after isoproterenol injection.

Tissue slides (5 μm), prepared from paraffin-embedded hearts collected at 6 months, were used for histological analysis. Collagen deposition was determined by Masson's staining (Sigma Aldrich) and expressed as percentage fibrotic tissue in 5 random fields at 40X magnification using ImageJ software (rsbweb.nih.gov/ij/). DNA damage was detected by the TUNEL assay (Apo-Tag plus, Millipore, Billerica, MA) and expressed as percentage of the TUNEL positive cardiomyocytes on the total cardiomyocytes over 10 random fields.

Statistical analysis was performed using the SPSS 15.0 package for Windows. Continuous variables were expressed as mean and standard error. The T test for paired data was used to compare variables before and after treatment. Kaplan Meier survival curves were constructed and compared among different groups. The T test for unpaired data was used to compare 2 groups. Two-tailed P values <0.05 were considered significant.

XRT induced no significant changes in LVEF at 3 days, and 1 and 4 months. Contractile reserve, however, was reduced in the XRT-treated mice as early as 3 days post-XRT ($p<0.05$ vs baseline and sham non-irradiated mice).

Between 4 and 6 months, 6 of the 12 (50%) XRT-treated mice died, while no deaths occurred in the 6 sham non-irradiated group (Figure, panel B)($p=0.049$). At 6 months, the surviving irradiated mice showed a significant reduction in LVEF (−14% [absolute reduction] and −20% [relative reduction], $p<0.05$ vs baseline and sham non-irradiated mice) and a further significant drop in contractility reserve at 6 months ($p<0.01$, for trend; $p<0.05$ vs baseline and vs sham non-irradiated mice, Figure, panels C-E) without LV dilatation or hypertrophy (Table). We detected no pericardial or valvular or regional wall motion

abnormalities suggestive myocardial infarction in either group at echocardiography or at postmortem examination.

XRT-treated mice had a 2-fold increase in myocardial interstitial fibrosis compared to sham non-irradiated mice at 6 months (p=0.014), reflecting XRT-induced injury. XRT-treated mice showed a 4-fold increase in TUNEL-positive cells (reflecting DNA fragmentation) compared to the sham non-irradiated mice, but the difference did not reach statistical significance.

Here we describe a mouse model of radiation-induced cardiomyopathy characterized by a latent phase of injury lasting several months in which the LVEF is normal but the mouse has significantly impaired contractile reserve, and a subsequent phase of reduced LVEF without evidence of LV enlargement of hypertrophy and in the presence of further impairment of contractile reserve, increased interstitial cardiac fibrosis and sudden death, a phenotype of overt cardiomyopathy. The absolute reduction in LVEF of 14% is likely highly significant, as in clinical trials using chemotherapy agents with cardiotoxicity an absolute decrease in LVEF of 5–10% is associated with an >4-fold increase risk of symptomatic heart failure and cardiac death [6].

Other animal models of XRT-induced cardiomyopathy have been reported [7]. In the most commonly used rat model, toxicity presents with acute pericarditis and effusion that may be lethal, but is generally not evident when doses <30 Gy are administrated [8].

A recent study confirmed that XRT increases the long-term risk of death due to heart failure in women that received XRT for the treatment of a left-sided breast cancer (>50% higher risk) than those treated for a right-sided breast cancer [9], and patients with XRT-induced cardiomyopathy have a reduction in peak oxygen consumptions of a degree consistent with severe heart failure [10].

The mechanisms leading to the late cardiomyopathy and sudden death are not completely understood but previous investigations suggest that an acute inflammatory response occurs early after radiation promoting tissue fibrosis [11]. The early decline in LV contractile reserve in our model is consistent with an early injury. From a clinical standpoint, the results of this study indicate that while the injury to the heart occurs immediately or very early after irradiation, a long latent clinical phase may occur. Consequently, many patients who have received chest irradiation in the past may have clinically latent disease and be at risk for late occurrence of heart failure or sudden death. It becomes clear that strategies are needed that are designed to prevent or limit the initial injury and/or the progression of the disease.

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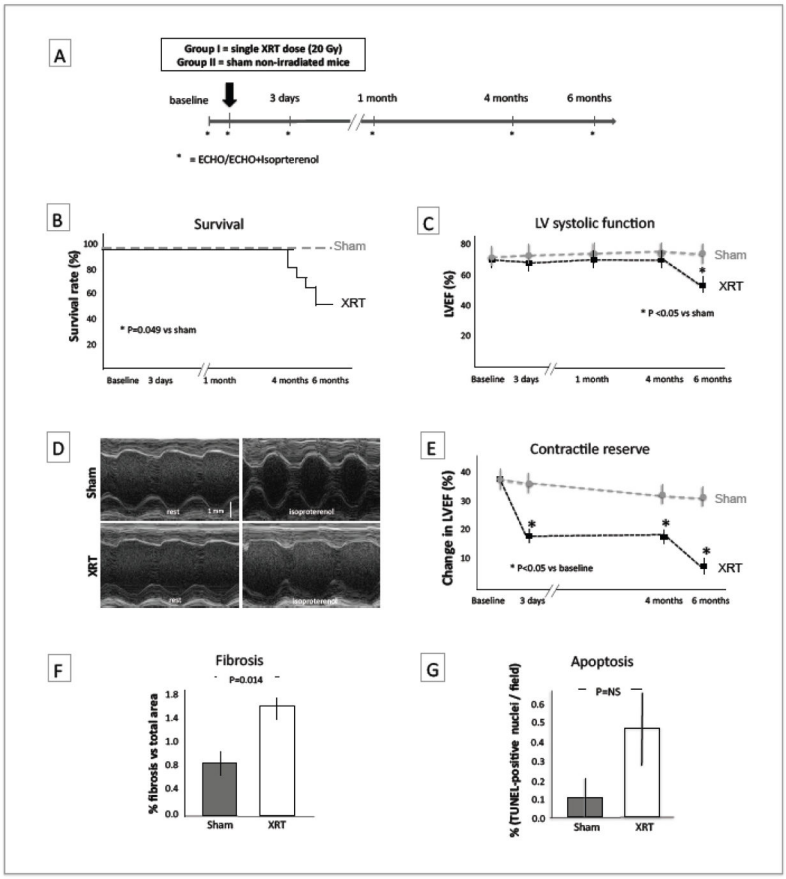

Figure.

The Experimental design is described in panel A: Group I (N=12) received a single thoracic radiation of 20 Gy at day 0, Groups II (N=6) sham non-irradiated mice were used as controls; an echocardiogram was performed at baseline (1 day prior to irradiation), 3 days, and 1, 4 and 6 months post irradiation; isoproterenol was administrated at 3 days, 4 and 6 months to measure contractile reserve. Panel B shows increased mortality in the XRT-treated group (p=0.049 vs sham non-irradiated). Panel C shows that XRT-treated mice had no significant change in LVEF up to 4 months and a significant drop in LVEF by 20% between 4 and 6 months (p<0.05 vs sham-non-irradiated). Panel D shows representative M-mode echocardiography recordings of the LV transverse mid-ventricular sections obtained before and after isoproterenol treatment in sham non-irradiated and XRT-treated mice. A M-Mode measurement of contractile reserve expressed as the change in LVEF measured before and after isoproterenol injection (a β-adrenergic agonist) is shown in panel E. The XRT-treated mice demonstrated a reduction in LV contractile reserve at 3 days, 4 and 6 months (p<0.05 vs sham-non irradiated). Interstitial myocardial fibrosis expressed as percentage of fibrotic areas on total area per field is shown in panel F, XRT-treated mice had a 2-fold increase in interstitial collagen deposition (p=0.014). Panel G shows a trend in the increment of $TUNEL^{+}$ nuclei (indicated with and asterisk) reflecting apoptotic DNA fragmentation (p=NS).

IP=intraperitoneal, isopt=isoproterenol, XRT= XRT-treated mice, LVEF=left ventricular ejection fraction.

Table

Echocardiographic parameters

XRT	**Baseline**	**6 months**	***Sham***	**Baseline**	**6 months**
LVEDD (mm)	3.5±0.2	3.6±0.2	LVEDD (mm)	3.5±0.2	3.6±0.3
LVEF (%)	68±4	56±4 *	LVEF (%)	68±3	67±4
LVM (mg)	73±4	83±5	LVM (mg)	76±4	96±5
HR (/min)	312±42	426±60	HR (/min)	412±32	426±30

HR=heart rate; LVEDD=left ventricular end-diastolic diameter; LVEF=LV ejection fraction; LVM=LV mass; XRT=irradiation

*P<0.05 vs baseline and vs sham.