Honors Thesis

The Role of Estrogen Receptor (ER) Signaling in Mitigating Radiation-Induced Cardiotoxicity (RIC) in Mouse Models Subjected to Total Body Irradiation.

Honors Thesis

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By

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Abstract

Radiation can be a significant concern for cancer survivors and radiation exposed to victims. Within the proposal document from Dr. Adam Chicco's lab, (Milestone 3) highlights a significant discrepancy in cardiac mortality rates between atomic bomb survivors and childhood cancer survivors. This difference likely stems from the nature of the exposure to total body irradiation (TBI) versus organ-specific (partial) radiation. A critical factor in TBI is the extreme radiosensitivity of the ovaries. Because the ovaries have a limited, non-renewable pool of oocytes (eggs), even low doses of radiation can cause premature ovarian failure (Adriaens et al.). This study investigates whether estrogen replacement can mitigate these effects when radiation is induced. Using a mouse model, estradiol capsules are used to maintain physiological estrogen levels following radiation exposure. Radiation exposure promotes cardiac scarring and stiffness, weakening the heart muscle's ability to pump blood effectively. To analyze these effects, echocardiography is utilized to visualize the anatomy of the heart. Consistent with the previous findings, estrogen replacement can help minimize pathological cardiac remodeling (Szabó et al.). While this research encompasses multiple analytical approaches, this paper focuses specifically on echocardiographic observations to understand the effects of RIC.

Introduction

Radiation is a recognized contributor to cardiovascular morbidity, yet the relationship between dose and cardiac outcomes is not fully understood. Researchers studied people who survived atomic bomb blasts and were exposed to radiation across their entire bodies. They found that for every unit of radiation exposure, measured in Grays (Gy), the risk of dying from heart disease increased by 14% (Shimizu et al.). In contrast, childhood cancer patients often require localized doses exceeding 15 Gy to manifest significant cardiac mortality trends. This discrepancy highlights that radiation-induced cardiotoxicity (RIC) was not determined solely by dose, but by factors such as radiation quality, dose rate, and organ-specific radiosensitivity.

Despite established guidelines, such as those from the International Commission on Radiological Protection (ICRP) that weighed reproductive organs as significantly more radiosensitive than the heart, the systemic interactions between these organs are poorly understood. Emerging evidence suggested that the progression of RIC might not have been significantly aggravated by the loss of estrogen signaling following radiation induced ovarian damage (ICRP). However, the precise role of this hormonal disruption in maintaining cardiovascular homeostasis post-exposure remained not fully understood. In this study, we investigated the impact of estrogen receptor (ER) signaling on cardiovascular health in an organism that has had radiation exposure. Through a collaborative experimental model, we exposed C3H/HeJ mice to 4 Gy of total body gamma-radiation and assessed whether estradiol supplementation could mitigate radiation-induced functional and molecular changes in the heart.

Radiosensitivity of Ovarian Follicle

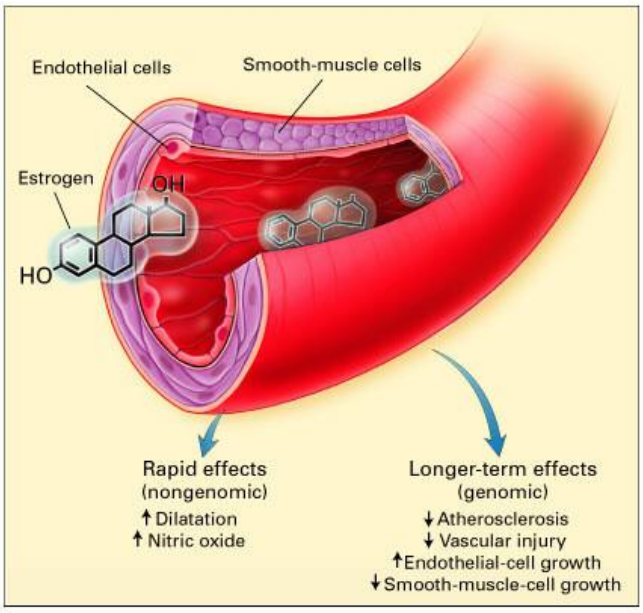
Ovaries do not react to radiation in a single, uniform way. Their sensitivity is greater than most other organs, and changes depending on the stage of the follicle and the species. Permanent ovarian failure typically requires high doses of radiation, 14 - 20 Gy in humans. However, the researchers acknowledged that biological impacts could occur at lower levels. In this study, the mice received a 4 Gy dose. This is lower than the doses required for permanent ovarian failure in humans; however, mice tend to be affected with lower Gy. This is crucial because using 4 Gy is likely to induce hormonal dysfunction and drop in estrogen production (Adriaens et al.). This project is investigating the signaling pathways (estrogen's role in the heart) rather than the mutational pathways (DNA damage in the ovaries). While we worry about DNA in ovaries and

the immediate physiological consequence, the hormonal drop is a factor that must happen to understand the impacts of estrogen.

Estrogen Receptor Signaling

Although the adrenal glands and adipose tissue release estrogen, too, it is mainly produced in the ovaries. Due to the larger production made by this organ, the radiation will be specific for the ovaries. In the biological male, the body converts small amounts of androgens (steroid hormone) produced in the testes and is a precursor for estrogen. Estrogen travels through the bloodstream until it reaches the part of the body where it binds to a protein, called an estrogen receptor (Figure 1.) There are estrogen receptors throughout the body, including the heart. Estrogen receptors in the heart play a critical role in cardiovascular protection by regulating vascular tone and decreasing oxidative stress. Activation of these receptors preserves mitochondrial function and maintains energy production while preventing heart tissue damage.

Figure 1.



The image above is to diagram when estrogen is following through the blood stream, it allows for the blood vessel walls to increase in dilation which supports smoother blood flow. (Mendelsohn and Kara

Estrogen are not just Sex Hormones

Estrogen levels can support a variety of systems in the body such as blood sugar levels, cholesterol levels, muscle mass, and strength. One of the main forms of estrogen in the body is estradiol (E2). E2 facilitates insulin secretion in the pancreas and helps the body monitor glucose availability, ensuring blood sugar levels stay balanced (Alemany). It supports decreasing cholesterol levels by increasing the number of receptors in the liver that can target Low density lipoprotein (LDL) particles from the blood to dispose of them as bile. Simultaneously, it boosts the production of good high density lipoprotein (HDL) particles and slows their breakdown, ensuring there is enough to protect arteries (Guetta and Cannon). Additionally, it increases the muscle's sensitivity to stimuli, such as exercise, which helps the body repair tissue and maintain muscle mass more effectively .

Particularly relevant to the current project, ER in the heart play a crucial, protective role in cardiovascular function. By regulating gene expression and cellular signaling pathways, ER can influence vascular tone, reduce oxidative stress, and support myocardial contractility. Estrogen hormones, particularly E2, serve a protective role in the body by maintaining the efficiency of mitochondria. They do this by keeping the energy-generating process known as oxidative phosphorylation tightly coupled, meaning energy is efficiently converted into cellular fuel (ATP) rather than being wasted. This process naturally keeps the production of harmful, stress-inducing molecules, called reactive oxygen species (ROS), low in the heart and other tissues. However, when the ovaries are removed, this protective hormone supply is lost. Without estrogen to keep the process running smoothly, the mitochondria become uncoupled and vulnerable. As a result, the body's natural antioxidant defenses drop, leaving the heart tissues susceptible to the toxic, damaging effects of estrogen degradation metabolites, which further increase ROS production and impair energy output (Uribe-Alvarez et al.).

Methods

Animal Model and Experimental Design

A total of 150 C3H/HeNCrl mice (75 male, 75 female) were purchased from Charles River at 7 weeks of age. Animals were acclimated for one week prior to the initiation of any experimental procedures.

Radiation Protocol

Chronic total body irradiation (TBI) was administered as a cumulative 4 Gy dose of gamma-rays protracted over 5 days to mimic a series of therapeutic radiation doses (e.g., for cancer treatment). Mice were in their cages within a specialized vivarium irradiator facility equipped with a bio-bubble for HEPA-filtered air circulation. Radiation dose was established through Milestone 3, given by a computational model representing the geometric and material properties of the facility, simulated using Monte Carlo N-Particle (MCNP) transport code.

Estrogen Replacement Therapy

Given the expectation that 4 Gy of gamma-ray exposure would damage ovaries and reduce E2 levels in female mice, we restored physiological E2 levels by implanting mice with silastic capsules containing 17 β-E2 dissolved in sesame oil (36 μg 17 β -E2/mL sesame oil) or sesame oil alone as the control group. To determine the effects of E2 on male mice following irradiation, E2 capsule were also implanted in a cohort of male mice. Capsule implantation was performed under isoflurane anesthesia. The capsule was inserted by a 0.5 cm incision made in the skin on the back of the neck and was closed via suture.

Following TBI, mice were assigned to one of four experimental cohorts:

(i) Male control (sesame oil)

(ii) Female control (sesame oil)

(iii) Male E2 Replacement (estradiol dissolved in sesame oil)

(iv) Female E2 Replacement (estradiol dissolved in sesame oil)

Echocardiography and Cardiac Assessment

Cardiac structure and function were assessed at baseline (pre-TBI) and at 15 days post-TBI (dpr). Transthoracic echocardiography was performed under light (2%) isoflurane anesthesia using a Phillips HD11 Ultrasound system equipped with a 15 MHz linear array transducer (sound wave). Short-axis two-dimensional (2D) images were recorded to evaluate Left ventricular (LV) chamber diameters in systole (LVIDs) and diastole (LVIDd), enabling calculation of LV fractional shortening as an index of LV contractile performance, described further below.

Fractional Shortening

Fractional shortening (FS) was measured as a mean of determining how much the left ventricle narrows during an average cardiac cycle. If the walls move inward significantly, the FS is high, indicating a greater force for muscle contraction. FS is determined by the difference between left ventricular end-diastolic internal diameter (LVIDd) and left ventricular end-systolic internal diameter (LVIDs) divided by LVIDd and multiplied by 100:

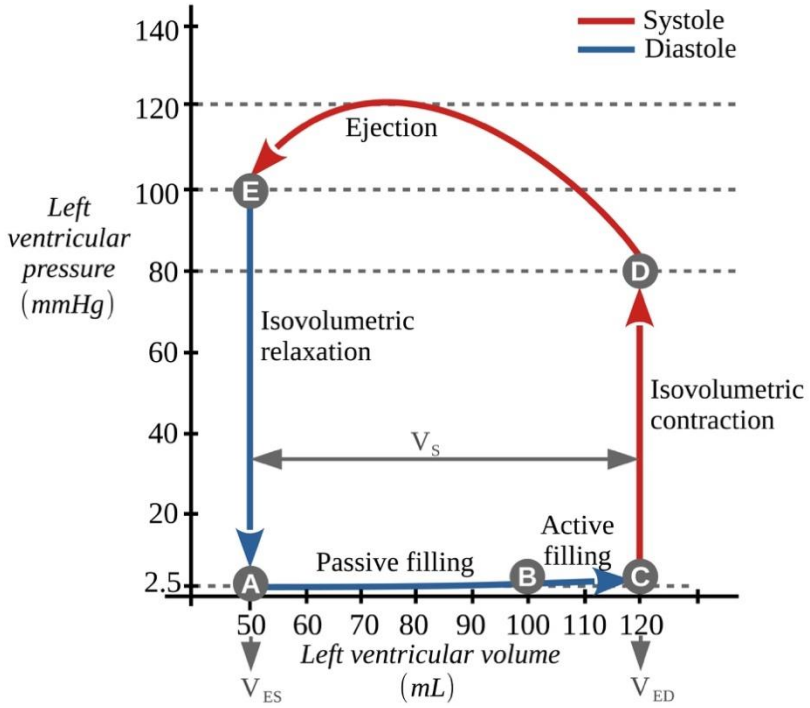
$$FS = \frac{LVIDd - LVIDs}{LVIDd} * 100$$

Ejection Fraction (EF)

Fractional shortening is a 2D expression of the common index of systolic function obtained from echocardiography in humans known as the Ejection fraction (EF)**,** which is the percentage of blood that leaves the left ventricle each time the heart beats. Since a healthy heart never fully empties, EF is calculated by taking the total volume of blood when the heart is full, subtracting what stays behind after a squeeze, and dividing that by the starting volume. Essentially, it turns the amount of blood moved into a percentage that shows how effectively the heart is pumping. To calculate for EF, it is the amount of blood in the heart when it's full (End-Diastolic Volume) and subtract what's left after the beat (End-Systolic Volume). That difference is your Stroke Volume.

$$EF = \frac{LVEDV - LVESV}{LVEDV} * 100$$

Figure 2.



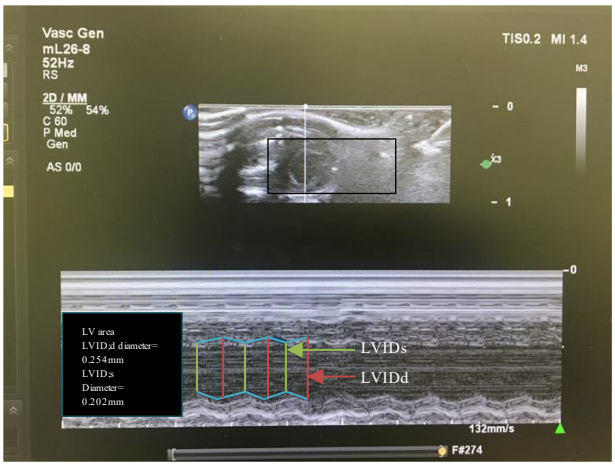
The image above diagrams the ejection fraction pressure changes by volume of the left ventricle within the heart measured in mmHg. During the filling phase (diastole), the pressure in the left ventricle is very low, typically around 5 to 12 mmHg, allowing it to expand and fill with blood. To eject that blood, the ventricle must then contract with enough force to exceed the pressure in the orta, usually reaching a peak systolic pressure of about 120 mmHg. (BrJCardiol)

Results

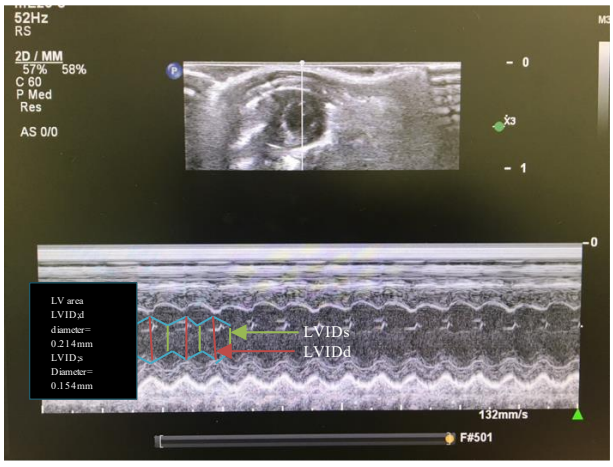
Figures 3 and 4 illustrate the two dimensional (2D)-guided M-mode images obtained during echocardiography on a mouse exposed to 4 Gy of gamma radiation protracted over 5 days (Fig 3) or maintained under non-irradiated control conditions for the same period (Fig 4). M-mode (motion mode) is an ultrasound imaging technique that displays the movement of heart structures over time along a single scan line. It provides a highly detailed, high-frequency view of the heart's internal dimensions and the movement of its valves and walls.

Figure 3.

Figure 4.



LVIDd diameter = 0.254mm
LVIDs diameter = 0.202mm
FS = 20.47%



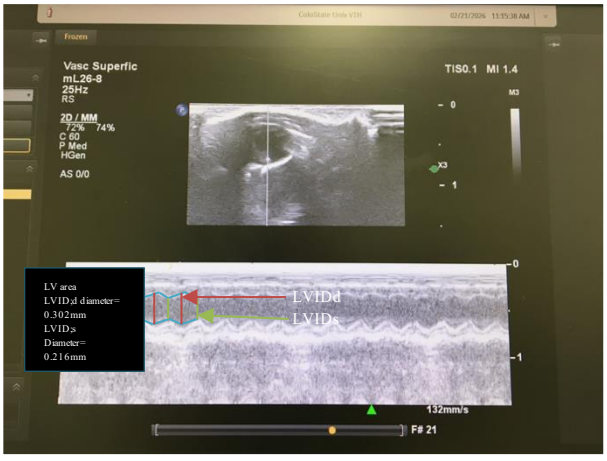
LVIDd diameter = 0.214mm
LVIDs diameter = 0.154mm
FS = 28.04%

Comparison between Mouse Sex with Control and Estrogen Replacement

Figure 5. and Figure 6. are both assessment of left ventricular systolic and diastolic function in male mice. They all have TBI and are taken 15 days post TBI. These figures illustrate echocardiographic assessments of left ventricular systolic function in mice using M-mode ultrasound imaging. In the images blow, all have been exposed to 4 Gy of gamma radiation.

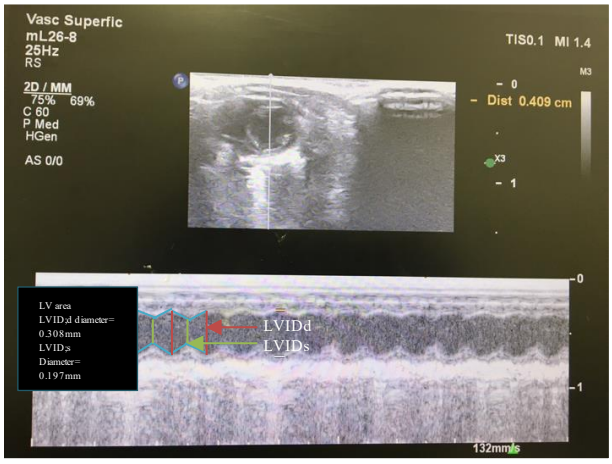
i) Male control (left) and Estrogen Replacement (right)

Figure 5.



LVIDd diameter = 0.302mm
LVIDs diameter = 0.216mm
FS = 28.10%

Figure 6.

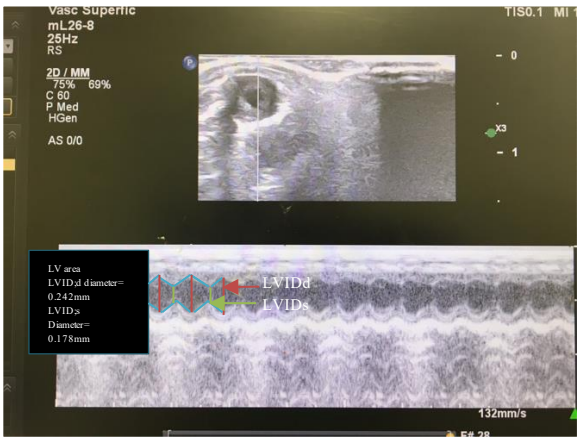


LVIDd diameter = 0.308mm
LVIDs diameter = 0.197mm
FS = 36.04%

Figure 7. and Figure 8. are both assessment of left ventricular systolic and diastolic function in female mice. They all have TBI and are taken 15 days post TBI. These figures illustrate echocardiographic assessments of left ventricular systolic function in mice using M-mode ultrasound imaging. In the images blow, all have been exposed to 4 Gy of gamma radiation.

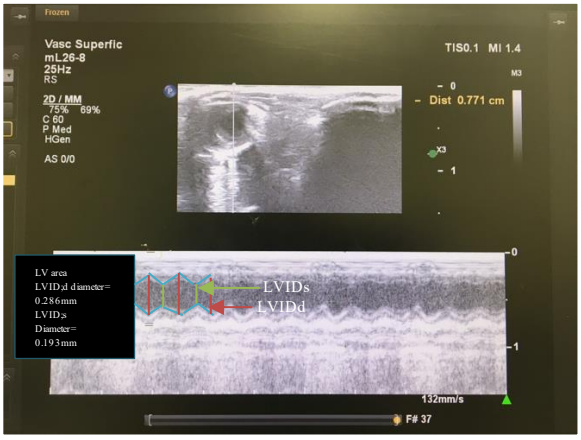
ii) Female control (left) and Estrogen Replacement (right)

Figure 7.



LVIDd diameter = 0.242mm
LVIDs diameter = 0.178mm
FS = 26.45%

Figure 8.



LVIDd diameter = 0.286mm
LVIDs diameter = 0.193mm
FS = 32.52%

Discussion

The aim of this ongoing study is to determine the role of estradiol treatment in mitigating radiation-induced cardiac dysfunction and evaluate the potential mechanisms of E2-mediated protection in both sexes. The preliminary data presented above indicates that ionizing radiation (IR) causes cardiac contractile dysfunction. The cardioprotective effect of E2 is observed in both male and female mice, suggesting a sex-independent mechanism of protection.

Given previous evidence that radiogenic injury to cardiac tissue results from IR-induced mitochondrial ROS production (Cao et al.), the Chicco Lab is also determining impacts of IR on cardiac mitochondrial ROS release from mice in this study. Ongoing experiments indicate that E2 significantly attenuates the release of reactive oxygen species (ROS) from cardiac mitochondria induced by 4 Gy of protracted gamma-ray exposure in both males and females in

this study. Preliminary data thus far has confirmed that 4 Gy of protracted gamma-ray exposure increases ROS release from cardiac mitochondria, which is significantly attenuated by E2 treatment in both male and female mice. Therefore, it is plausible that the greater FS in E2-treated mice following IR in this study could be explained by less cardiac oxidative stress and/or better mitochondrial function. These findings offer potential insight to how estrogen may protect the heart from radiation exposure, thereby justifying the potential utility of estrogen replacement as a means of reducing cardiovascular risk in individuals previously exposed to ionizing radiation.

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