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Research Article

Assessment of Cardiotoxicity in Mice Following Thoracic Radiotherapy and Systemic Stem-Cell Derived Extracellular Vesicle Treatments

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Abstract

Aim: Radiotherapy is an important treatment modality against breast and thoracic cancer, bringing significant improvement in tumor control and survival. Nevertheless, it also results in variable degrees of cardiac exposure to ionizing radiation. Resultant radiation-induced heart disease (RIHD) typically manifests at protracted post-irradiation times and can involve a wide range of underlying pathologies including heart failure. Past work from our group has identified an intervention involving systemic administration of human embryonic stem cell (hESC)-derived extracellular vesicles (EV) shown to resolve radiation-induced lung disease (RILD). However, potential treatment-associated complications to the heart were not investigated. Using a similar EV-based treatment paradigm, we conducted a safety to study evaluate the potential impact of systemically administered EV on cardiac functionality after a single image-guided dose (14 Gy) of thoracic irradiation.

Method: Separate cohorts of control (CONT: n=8, no irradiation, no EV), irradiated (IR: n=8, 14 Gy + 4 weekly vehicle injections) and irradiated and EV treated (IR + EV: n=8, 14 Gy + 4 weekly EV injections) mice were implemented in this study. For the irradiated groups, mice were given 4 systemic (retro-orbital) vehicle injections +/- EV the week of and for 3 weeks after IR. Echocardiography follow-up was used to measure E/A ratio, ejection fraction (EF), shortening fraction (SF), left ventricular mass (LV mass) and end diastolic volume (EDV) 20-weeks following IR.

Results: Radiation exposure caused a significant drop in E/A ratios compared with mice that did not receive radiation, with non-significant changes in other key indicators of cardiac function. EV treatments were not found to have an observable effect on the E/A ratio or any other indices of cardiac function among the different cohorts.

Conclusions: Irradiated mice had lower E/A ratios, but other indicators of cardiac function were unchanged, suggesting that thoracic exposures compromised diastolic function without impacting EF. 20 weeks post exposure. Diastolic dysfunction was most likely linked to increased radiation-induced fibrosis and myocardium stiffness. Importantly, EV treatments were not observed to adversely impact cardiac function, pointing to the safety of this potential intervention for RILD.

INTRODUCTION

Radiation induced heart disease (RIHD) is a serious complication of radiotherapy. Despiote the use of targeted radiotherapy to treat breast cancer, lymphoma, and lung cancer, RIHD remains one of the most critical constraints to treatment. Clinical manifestations of RIHD include cardiomyopathy, heart failure, ischemic changes, valvular disease, arrythmias and pericarditis [1]. With more and more cancer patients showing long term survival following their treatment, such debilitating side effects significantly decrease their quality of life. The higher the radiation dose the more morbidity of RIHD [2].

The main strategy to prevent cardiotoxicity in the past has consisted in radiation protocols and technologies to avoid including the heart in the radiation field, such as inspirational breath holding [3], thereby minimizing organ motion and inadvertent radiation dose delivered to the heart. More modern radiotherapy treatments now allow better targeting of the tumor and gated heart avoidance, although such techniques do not

completely protect the heart from radiation exposure. To date, there has been no approved drug to treat or prevent radiation induced cardiac toxicity. Experimental therapies in the past have included antioxidant or anti-inflammatory treatment before or shortly after exposure [4]. However, this is not an approved treatment for RIHD [2]. While some therapies have been shown to mitigate RIHD, none of them offer the potential of stem cell therapy to forestall the disease by counter-acting cell damage, loss and recovering homeostasis. In addition, stem cell therapy has shown to provide support to surrounding host tissue [5]. More recently there has been advances in the field of cardiac stem cell therapy [6], but the investigation of such approaches for RIHD has not been well studied.

Contrary to stem-cell therapy, extracellular vesicle (EV)-based therapies have the advantage of not harboring the risk of downstream tumor formation and do not necessitate immune suppression. Recently, human embryonic stem-cells EV-based therapies developed in our group have been showed to significantly prevent the occurrence of radiation-induced toxicities in the brain [7,8] and in the lung [9], improving overall survival and preventing the development of lung fibrosis. While their full mode of action is still under investigation, the functional transfer of their diverse bioactive cargo (nucleic acids, lipids, proteins organelle fragments) following their endocytosis into target cells has been identified to promote anti-inflammatory and regenerative processes. One proposed mechanism includes downregulating TLR-4 signaling pathway [10]. Another study showed a decrease in effector CD8+ and CD4+ T cells in a mouse model [11]. Due to their potential to prevent radiation-induced normal tissue toxicity in a safe, non-immunogenic manner, EV-based therapies could be implemented in clinical trials for the management of multiple radiation-induced side effects. However, functional outcomes in the heart following thoracic irradiation and stem-cell derived EV-treatments were not evaluated in our previous or other studies. Here we describe our study designed to test the hypotheses of 1) would thoracic radiation cause a certain level of heart dysfunction, and 2) would stem cell-derived EV further confound RIHD.

MATERIALS AND METHODS

Stem cell culture and isolation of EV

Growth, culturing, and maintenance of human embryonic stem cells was approved by the Institutional Human Stem Cell Research Oversight (HSCRO, #2007-5629) and Institutional Biosafety (IBC) Committees. The hESC line H9 (WA09 Wicell Research Institute, Inc., Madison, WI) was cultured and expanded in Nutristem XF medium (Biological Industries, Cat# 05-100-1A; Cromwell, CT) in a humidified incubator (5% CO2, 37°C). Six well tissue culture plates (Corning, NY) were coated with Vitronectin XF diluted in Cell Adhere Dilution Buffer (STEMCELL Technologies, Cat. # 07180; Vancouver, CA). Cells were passaged every 4-6 days with manual selective passaging technique using an EVOS4 microscope. Conditioned medium was collected from cells between passage 45 and 60. Cell pluripotency was confirmed by staining for Oct3/4 and Nanog markers. The cells were shown regularly to test negative for mycoplasma with MycoAlert Mycoplasma Detection Kit (Lonza, Cat# LT07-118; Basel, Switzerland).

For the harvest of conditioned media from hESC, culture medium was changed every day with 2 mL from plating to 50% confluence. At 50% confluence, the medium is replaced with 4 mL per well and conditioned medium harvested from morphologically and physiologically optimal colonies for three days until 80% confluency was achieved. Yield from a single 6 well plate is 48 mL and cell surface area of colonies in one 6 well plate at 50% and 80% confluence is 28.5 cm^2 and 45.6 cm^2 respectively. Conditioned medium is briefly stored at 4°C until ~420 ml total volume is obtained, sufficient for most applications described herein.

For the isolation of EV, pooled stocks of conditioned media collected over the duration cell culturing were stored at 4°C before ultracentrifugation. Details describing EV isolation via ultracentrifugation have been described [8]. Briefly, while maintaining sterility, conditioned media is spun at 2500 x g at 4°C for 20 min to remove subcellular debris and the supernatant is bulk filtered (0.45 μm). The filtrate is transferred to 70 mL polycarbonate ultracentrifuge bottles (Beckman) and spun at 100,000 x g at 4°C for 90 min. The supernatant is discarded, and pelleted EV are collected in PBS. EV from six isolations are typically pooled into smaller polycarbonate ultracentrifuge bottles for ease of collection and washed with PBS, pelleted once more at 100,000 x g at 4°C for 120 min. Concentrated EV are resuspended in small volumes of PBS, quantified and characterized using a Zetaview instrument (ZetaView PMX 110; Meerbusch, Germany) with yields varying between 1 x 10^{9-12}/ml depending on cell type and media volumes with a typical size distribution of 100 ± 55 nm (diameter).

Irradiations and EV treatments

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California Irvine and performed within institutional guidelines. Male C57BL/6J mice (n=24) were bred in our laboratory and involved in the study at the age of 22-24 weeks.

Mice were anesthetized (2% isoflurane) and received local thoracic irradiation using a SmART irradiation system (Precision X-ray). The prescribed dose was determined at 10 mm depth with a 15 mm circular collimated field according to previous depth dose measurements in a solid water phantom. Irradiations were performed at 225 kV, 20 mA, with a 0.3 mm copper filter and delivered after fluoroscan imaging to position the mice at the treatment isocenter. Whole thorax irradiation was performed on mice with two opposite vertical beams delivering 14 Gy in total, including the whole lungs and heart in the irradiation field.

EV treated groups received four retro-orbital injections of 10^{10} hESC-derived EV. All injections (vehicle and EV) were performed under isoflurane anesthesia. Non-irradiated animals

were divided into control (0 Gy, no EV; n=8), radiation only arm (14 Gy + 4 weekly vehicle injections; n=8), radiation + EV treatment (14 Gy + 4 weekly injections of EV; n=8).

Cardiac ultrasonography

Echocardiography was performed in 3 cohorts to investigate the impact of sub-volume heart irradiation and EV therapies. Mice in each cohort were imaged at 20 weeks post-IR.

High-resolution ultrasonography was used to assess *in vivo* cardiac function in the 3 cohorts. On the day of ultrasonography, mice were anesthetized with 1.5–2% isoflurane and shaved to remove hair on the thorax and abdomen. Animals were then placed supine on a heated platform that monitored the animal's respiration rate and body temperature. For echocardiography, mice were scanned using a Prospect T1 imaging system (S-Sharp, New Taipai City, Taiwan) with a PB506e (30-50 MHz) transducer. Echocardiographs were obtained in the short axis M-mode at the mid-left ventricular level. Pulsed-wave Doppler was used to determine mitral valve E and A velocities in a four-chamber view of the heart.

The Scintica software cardiac package was used to obtain echocardiographic parameters of ejection fraction from three M-mode scans per animal. The vascular package was used to assess mitral valve E and A velocities from three consecutive wave patterns per Pulsed-wave Doppler scan, in up to three scans per animal. Both ultrasonography and subsequent data analysis was performed in a blind fashion. Three images were acquired for each animal: 1) parasternal Long Axis of the LV – B-mode; 2) parasternal Short Axis of the LV – B-mode and M-mode and 3) apical 4 chamber view to get Mitral Valve flow – PW Doppler (E/A ratio).

From these images, the following cardiac function parameters were measured: E/A (Power-Doppler analysis of mitral valve flow), ejection fraction (EF), shortening fraction (SF), left ventricular mass (LV mass), end diastolic volume (EDV) measured via mode and short/long axis compiled measurement.

Organ sampling and histopathology

Animal euthanasia was performed with CO_2 24 weeks post irradiation. The heart was collected, fixed in FineFIX (#84-1717-00/Biosystems) and kept at 4°C before being paraffin embedded and cut into 4 µm sections. The sections were stained with a solution of hematoxylin-eosin (HE) and examined using an inverted brightfield microscope. Cardiomyocyte sizes were measured using Aperio ImageScope software version 12.4.3.5008 under a uniform, calibrated field size. Individual cardiomyocytes were circumscribed to calculate length and area of a minimum of sixty measurements per animal.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism (v9) software. Echocardiography measurements were performed 3 times on each animal (n= 8 group) and presented as mean ± SD. Cardiomyocyte measurements were performed on a minimum of 60 cells per animal (n=4 / group) and presented as mean ± SD. All data were analyzed using Kruskal-Wallis one-way ANOVA followed by Mann-Whitney U test. Data in the text are presented as means ± SD, and all analyses considered a value of $p \leq 0.05$ to be statistically significant.

RESULTS

In this study, mice received a single 14 Gy dose total thorax irradiation followed by systemic injections of hESC-derived extracellular vesicles (Figure 1). To obtain critical readouts of cardiac function from each of the treatment groups echocardiography was performed.

Measurements of E/A ratios in irradiated and non-irradiated animals showed a significant impact of irradiation on heart function. Lower E/A ratios were observed in irradiated mice compared to non-irradiated animals (1.405 ± 0.174 vs. 1.069 ± 0.284; p=0.019), characterized by an inverted E/A ratio (Figure 2). However, there was no statistically significant difference in E/A ratios between radiation alone arm vs. the EV treated group (1.069 ± 0.284 vs. 1.324 ± 0.643; p=0.554). Moreover, no significant difference in EF, SF, LV mass, or EDV was observed between any cohort [Table 1].

To further evaluate any possibly consequences of the treatments on overt cellular structure and size, a histological analysis was performed. At the level of brightfield microscopy (40x, 60x) no obvious changes in subcellular structure were apparent among the different cohorts. Furthermore, no difference in cardiomyocyte length or volume (Figure 3) was observed between all cohorts. No influence of radiation was observed 24-weeks post-irradiation on the length and area of the cardiomyocytes in the left ventricular wall. Animals treated with EV injections showed similar cardiomyocytes shape and size when compared to the non-irradiated or to the RT + vehicle groups.

DISCUSSION

Radiation-induced normal tissue toxicity is a prime determinant that governs treatment plans specifics that include, total dose, dose/fraction and number of fractions, factors that are evaluated based on multiple disease and patient parameters. In the end, whether for curative intent, recurrence, or palliation, delivering external beam irradiation remains an imperfect science, despite major advances in conformality. While thoracic irradiation procedures are mainly used to treat breast and lung tumors, stereotactic ablative techniques have now emerged as viable treatment options for ventricular tachycardia (VTAC)

Table 1: Cardiac function parameters (mean ± SD)

	No RT	RT + Vehicle	RT + EV
E/A ratio	1.405 ± 0.174	1.069 ± 0.284	1.324 ± 0.644
Ejection Fraction (EF) (%)	47.43 ± 10.47	53.99 ± 8.99	61.18 ± 10.63
Shortening Fraction (SF) (%)	23.65 ± 6.06	27.37 ± 5.45	32.65 ± 7.42
Left Ventricular Mass (LVM) (mg)	153.3 ± 49.43	149.4 ± 40.61	155.4 ± 33.25
End Diastolic Volume (EV) (µL)	59.28 ± 21.24	47.30 ± 18.25	58.41 ± 17.87

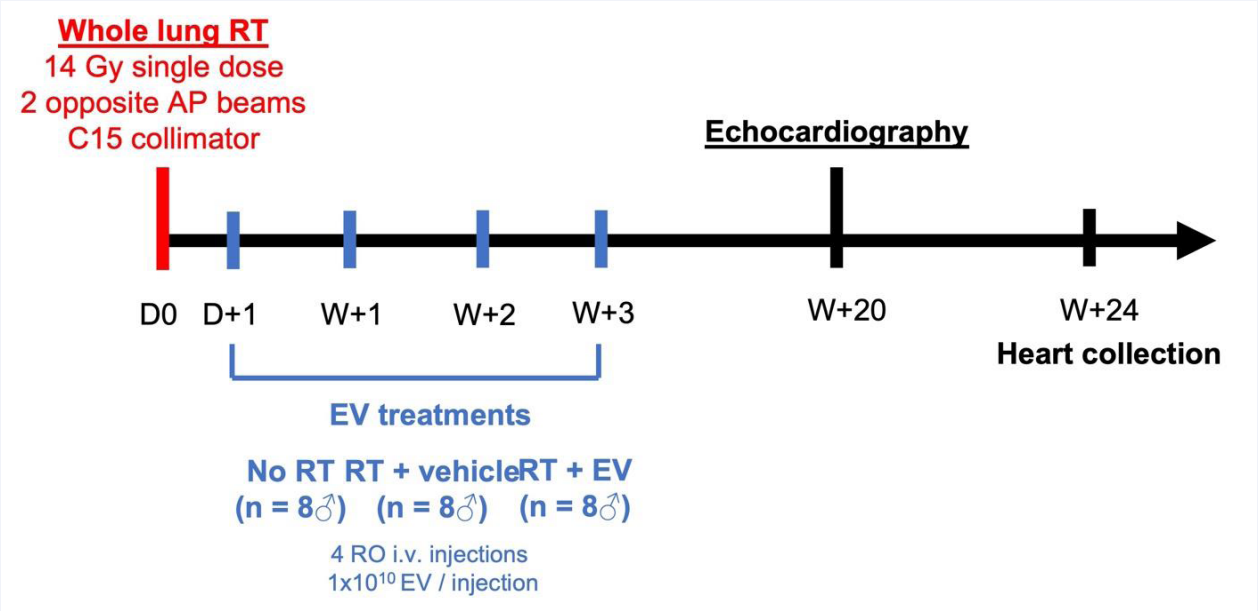

Figure 1 Study design.

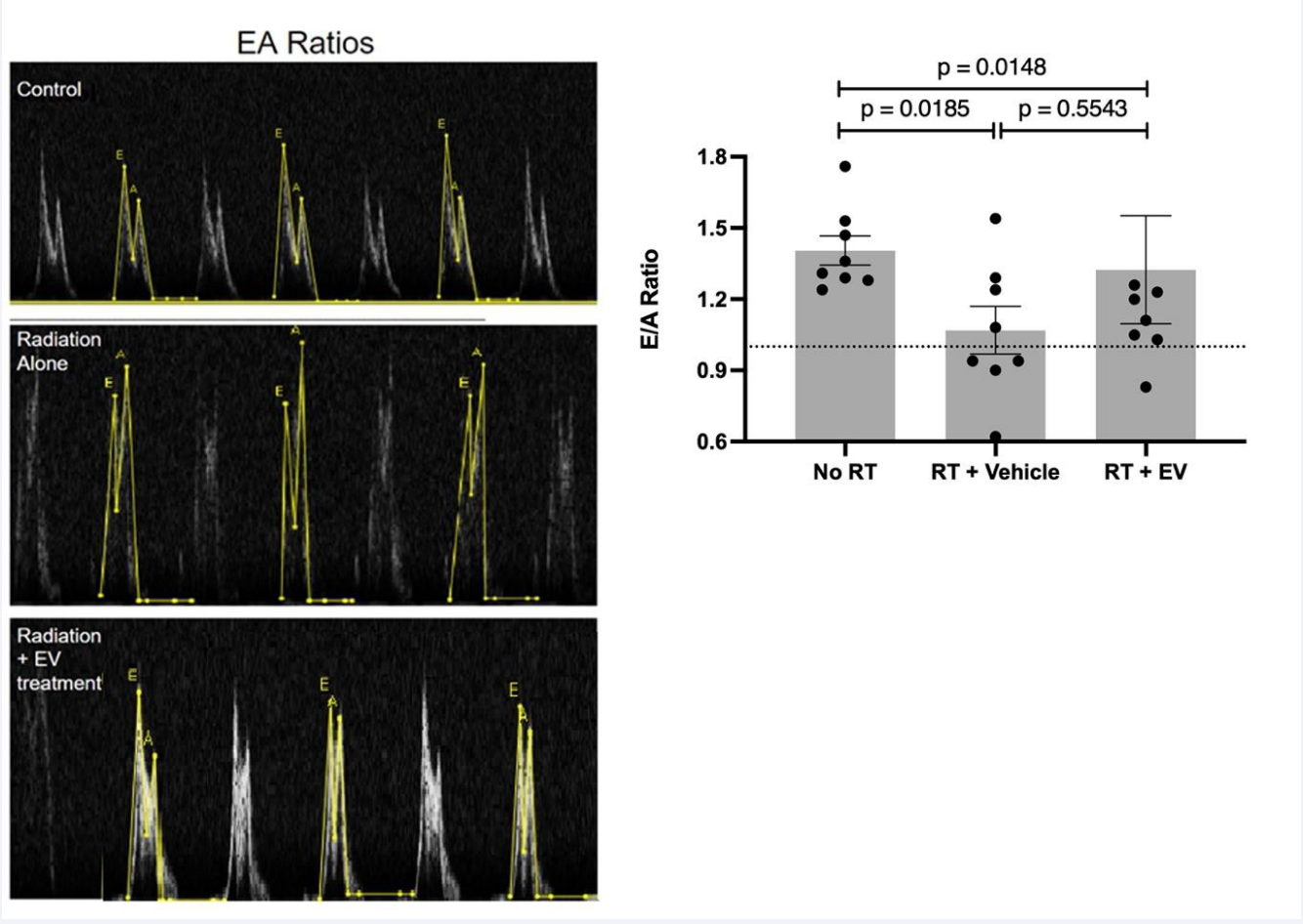

Figure 2 E/A ratios of mice 20 weeks post exposure to sham irradiation (No RT) or 14 Gy single dose to the whole thorax and treatment with 4 i.v. injections of hESC-derived EV (RT+ EV) or PBS (RT + Vehicle). Mean ± SD.

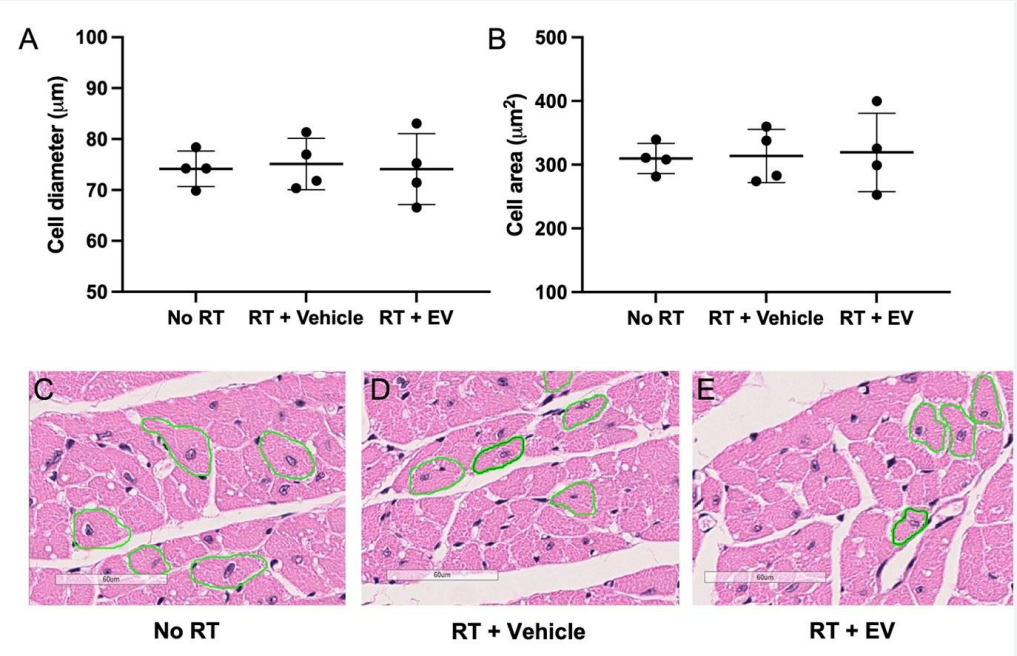

Figure 3 Cell diameter (μm) (A) and cell area (μm2) (B) of cardiomyocytes 24 weeks post exposure to a sham irradiation (No RT) or 14 Gy single dose to the whole thorax and treatment with 4 i.v. injections of hESC-derived EV (RT+ EV) or PBS (RT + Vehicle). Mean ± SD. Individual cardiomyocytes were circumscribed to calculate length and area of a minimum of sixty measurements per animal on non-irradiated controls (No RT; C), animals irradiated and treated with vehicle (RT + Vehicle; D) and animals irradiated and treated with EV (RT+EV; E).

when more traditional treatment options are no longer viable. In each case there are variable risks for cardiac dysfunction, largely dependent on the total dose of cardiac exposure. Apart from VTAC, and despite treatment planning efforts to minimize cardiac exposure, partial volume irradiation of the heart is often unavoidable.

Given this backdrop, significant findings have documented the adverse effects of inadvertent cardiac exposure resulting from thoracic irradiation procedures, and understandably, efforts have been investigated for mitigating radiation-induced toxicities to the heart. Stem cell- and EV-based therapies have met with success where in vitro approaches have documented increased contractility of cardiomyocytes, reduced proinflammatory cytokine signatures and improved mitochondrial bioenergetics. Promising results have also been found in various pre-clinical models, that have largely involved direct intracardiac injections of select stem cells or cell-derived EV and/or exosome preparations. Regenerative repair of cardiac tissue injured from infarct, targeted LPS administration, disease and systemic chemotherapy have pinpointed several mechanisms of action and provided the rationale for clinical trials and the use of iPSC-derived myocytes for personalized risk diagnoses and treatment outcomes. In one study of rodents post cardiac infarct, hESC derived cardiomyocytes showed to partially muscularized damaged tissue and improve function [12]. To date however, these approaches have not been extended to the prospective diagnosis and/or resolution of late RIHD.

Our group has been successful at implementing a variety of stem cell- and EV-based approaches for the resolution of functional and molecular deficits resulting from cranial and more recently thoracic irradiation. Initial strategies implementing direct intracranial transplantation of stem cells in immune-compromised rodent models have since evolved to the systemic administration of stem-cell derived EV in immune-competent mice, promoting the translational feasibility of these less invasive and immune tolerable treatments. With these more clinically amenable approaches, systemic EV treatments were demonstrated to resolve radiation-induced cognitive dysfunction, reduce neuroinflammation, preserve dendritic architecture and mitigate pneumonitis. Of note was that the reduction of these serious normal tissue toxicities could be accomplished using a single systemic injection of stem-cell derived EV, prompting a detailed proteomic evaluation of the exosome cargo. While a wealth of potentially protective protein complexes was identified within hESC-derived exosomes, including a full complement of proteins involved in mitochondrial electron transport, DNA damage and repair, various antioxidants, nuclear pore components and NAMPT, their potential beneficial or adverse impact on cardiac outcomes was not investigated. Here, our primary focus was to evaluate the safety of systemic hESC-derived exosome treatments on cardiac function, previously shown to be beneficial for RILD, and secondarily, to ascertain whether any mitigation of RIHD might be found.

In this study we showed that C57Bl/6J male mice irradiated with a single 14 Gy dose on the thorax had lower E/A ratios 20

week-post-exposure compared to non-irradiated mice. However, there was no difference in EF between any group, rather suggesting that heart irradiation causes diastolic dysfunction with preserved EF, as already described in the literature [13]. Another study showed dose dependent relationship with the level of heart function and amount of radiation [14]. One explanation for diastolic dysfunction post-irradiation is the development of pathological fibrosis, a process driven by TGFb and inducing tissue stiffness and loss of function [15]. In human patients with heart failure and preserved ejection fraction (HEpEF), myocardial stiffness has been linked to the accumulation of extracellular matrix, including fibrillar collagen and cardiomyocyte titin [16]. Increasing tissue stiffness leads to impaired filling and relaxation and thus and lower E/A ratio. The E/A ratio is calculated by doppler flow and represents peak velocity in early diastole, the "E" wave, and in late diastole caused by atrial contraction, "A" wave. Thus, there would be less early diastolic filling in a stiff and non-compliant heart muscle leading to a smaller "E" wave and smaller E/A ration. Smaller E/A ratio has been linked to diastolic dysfunction [17]. The same experiments performed on female mice, at a similar dose and at the same time point, showed no significant difference in cardiac function between the irradiated and unirradiated groups (data not shown). These results correlate with the well described protective action of estrogen and estrogen receptors against cardiovascular disease in females [18], highlighting the importance of further investigating sex-dependence of radiation-induced toxicities.

Our results show that hESC-derived EV injected post-irradiation did not prevent nor worsen diastolic dysfunction compared to irradiated animals only, up to twenty-four weeks post-exposure. While our prior results showed systemic benefits of EV that could ameliorate RILD, current efforts were unable to confirm any benefits of our EV regimen on RIHD. Nevertheless, it has been shown that radiation damage to the heart can take years or even decades to manifest [19], suggesting that changes in the timing and/or dosing of EV could be optimized to forestall these late effects.

Challenges for applying stem cell therapy for RIHD include identification of optimal cell types, route of administration, number of cells and timing of therapy [20]. Here we show that a single systemic injection of 10^{10} hESC-EV, proven to be efficacious to prevent RILH [9] does not worsen RIHD. Moreover, no further cardiac injury was observed in animals treated with higher EV doses over the course of four weeks, suggesting an overall safety of hESC-derived EV treatments. Furthermore, echocardiography was used to assess cardiac function, but more sensitive methods might be necessary to evaluate the effects of EV therapy, such as MRI or photon emission computed tomography [21]. Future studies may also include echocardiography and cardiac MRI at various time points post-treatment to pinpoint more precisely the onset of diastolic dysfunction. In addition, longer term follow-up would be fruitful to assess longer-term cardiac complications and how they track with overall survival and quality of life metrics [22]. Notwithstanding, current findings point to the promise of EV-based treatments for resolving certain indications of radiation-induced normal tissue injury, and at least to date, have not uncovered undesirable complications.

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AUTHORS CONTRIBUTION

Conception and design: PMG, CLL, ASB

Data acquisition: RB, ASB

Data analysis: RB, PMG

Funding acquisition: CLL, PMG

Project administration: CLL

Supervision: CLL, ASB

Writing: PMG, CLL, RB, ASB

Availability of data and materials

All data are available in the main text.

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ETHICAL APPROVAL

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California Irvine and performed within institutional guidelines.

REFERENCES

1. Sárközy M, Varga Z, Gáspár R, Gergő Szűcs, Mónika G Kovács, Zsuzsanna ZA Kovács, et al. Pathomechanisms and therapeutic opportunities in radiation-induced heart disease: from bench to bedside. Clin Res Cardiol. 2021; 110: 507–531.
2. Wang H, Wei J, Zheng Q, Lingbin Meng, Ying Xin, Xia Yin, et al. Radiation-induced heart disease: a review of classification, mechanism and prevention. Int J Biol Sci. 2019; 15: 2128–2138.
3. Desai N, Currey A, Kelly T, Bergom C. Nationwide Trends in Heart-Sparing Techniques Utilized in Radiation Therapy for Breast Cancer. Adv Radiat Oncol. 2019; 4: 246–252.
4. Slezak J, Kura B, Babal P, Miroslav Barancik, Miroslav Ferko, Karel Frimmel, et al. Potential markers and metabolic processes involved in the mechanism of radiation-induced heart injury. Can J Physiol Pharmacol. 2017; 95: 1190–1203.
5. Ren G, Chen X, Dong F, Wenzhao Li, Xiaohui Ren, Yanyun Zhang, et al. Concise Review: Mesenchymal Stem Cells and Translational Medicine: Emerging Issues. Stem Cells Transl Med. 2012; 1: 51–58.
6. Ptaszek LM, Mansour M, Ruskin JN, Chien KR. Towards regenerative therapy for cardiac disease. Lancet. 2012; 379: 933–42.
7. Leavitt RJ, Limoli CL, Baulch JE. miRNA-based therapeutic potential of stem cell-derived extracellular vesicles: a safe cell-free treatment to ameliorate radiation-induced brain injury. Int J Radiat Biol. 2019; 95: 427–435.

8. Baulch JE, Acharya MM, Allen BD, Ning Ru, Nicole N Chmielewski, Vahan Martirosian, et al. Cranial grafting of stem cell-derived microvesicles improves cognition and reduces neuropathology in the irradiated brain. Proc Natl Acad Sci U S A. 2016; 113: 4836–4841.

9. Montay-Gruel P, Zhu Y, Petit B, Ron Leavitt, Mike Warn, Erich Giedzinski, et al. Extracellular Vesicles for the Treatment of Radiation-Induced Normal Tissue Toxicity in the Lung. Front Oncol. 2021; 10: 602763.

10. Li X, Liu L, Yang J, Yonghui Yu, Jiake Chai, Lingyan Wang, et al. Exosome Derived From Human Umbilical Cord Mesenchymal Stem Cell Mediates MiR-181c Attenuating Burn-induced Excessive Inflammation. EBioMedicine. 2016; 8: 72–82.

11. Cosenza S, Toupet K, Maumus M, Patricia Luz-Crawford, Olivier Blanc-Brude, Christian Jorgensen, et al. Mesenchymal stem cells-derived exosomes are more immunosuppressive than microparticles in inflammatory arthritis. Theranostics. 2018; 8: 1399–1410.

12. Zhu W-Z, Hauch KD, Xu C, Laflamme MA. Human embryonic stem cells and cardiac repair. Transplant Rev. 2009; 23: 53–68.

13. Saiki H, Moulay G, Guenzel AJ, Weibin Liu, Teresa D Decklever, Kelly L Classic, et al. Experimental cardiac radiation exposure induces ventricular diastolic dysfunction with preserved ejection fraction. Am J Physiol Circ Physiol. 2017; 313: H392–H407.

14. Zhang J, Yu C, Yao BW, Hui Wang, Li Zhao, Xin Ping Xu, et al. Dose-dependent Cardiac Dysfunction and Structural Damage in Rats after Shortwave Radiation. Biomed Environ Sci. 2020; 33: 603-613.

15. Desai MY, Windecker S, Lancellotti P, Jeroen J Bax, Brian P Griffin, Oren Cahlon, et al. Prevention, Diagnosis, and Management of Radiation-Associated Cardiac Disease. J Am Coll Cardiol. 2019; 74: 905–927.

16. Zile MR, Baicu CF, S. Ikonomidis J, Robert E Stroud, Paul J Nietert, Amy D Bradshaw, et al. Myocardial Stiffness in Patients With Heart Failure and a Preserved Ejection Fraction. Circulation. 2015; 131: 1247–1259.

17. Schumacher A, Khojeini E, Larson D. ECHO parameters of diastolic dysfunction. Perfusion. 2008; 23: 291–296.

18. Murphy E, Kelly DP. Estrogen signaling and cardiovascular disease. Circ Res. 2011; 109: 687–696.

19. Desai MY, Jellis CL, Kotecha R, Johnston DR, Griffin BP. Radiation-Associated Cardiac Disease. JACC Cardiovasc Imaging. 2018; 11: 1132–1149.

20. Benderitter M, Caviggioli F, Chapel A, Robert P Coppes, Chandan Guha, Marco Klinger, et al. Stem Cell Therapies for the Treatment of Radiation-Induced Normal Tissue Side Effects. Antioxid Redox Signal. 2014; 21: 338–355.

21. Hoover-Plow J, Gong Y. Challenges for heart disease stem cell therapy. Vasc Health Risk Manag. 2012; 8: 99-113.

22. Walls GM, O'Kane R, Ghita M, Refik Kuburas, Conor K McGarry, Aidan J Cole, et al. Murine models of radiation cardiotoxicity: A systematic review and recommendations for future studies. Radiother Oncol. 2022; 173: 19–31.