

Review Article

Preclinical Research into Basic Mechanisms of Radiation-Induced Heart Disease

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Radiation-induced heart disease (RIHD) is a potentially severe side effect of radiotherapy of thoracic and chest wall tumors if all or part of the heart was included in the radiation field. RIHD presents clinically several years after irradiation and manifestations include accelerated atherosclerosis, pericardial and myocardial fibrosis, conduction abnormalities, and injury to cardiac valves. There is no method to prevent or reverse these injuries when the heart is exposed to ionizing radiation. This paper presents an overview of recent studies that address the role of microvascular injury, endothelial dysfunction, mast cells, and the renin angiotensin system in animal models of cardiac radiation injury. These insights into the basic mechanisms of RIHD may lead to the identification of targets for intervention in this late radiotherapy side effect.

1. Introduction

The worldwide number of long-term cancer survivors is growing fast with ongoing improvements in cancer therapies [1, 2]. However, long-term cancer survivors may suffer from late side effects of cancer therapy. One of these late side effects is radiation-induced heart disease (RIHD), which may occur after radiotherapy of thoracic and chest wall tumors whenever all or part of the heart is situated in the radiation field. RIHD has been described to occur, for instance, among survivors of Hodgkin's Disease [3, 4] and breast cancer [5, 6]. Radiotherapy planning has undergone many improvements over the last decades, with modalities such as Intensity-Modulated Radiation Therapy (IMRT), image-guided radiation therapy, and proton therapy, leading to reduced exposures of the heart. Nonetheless, recent studies indicate that problems may persist. For instance, patients with Hodgkin's Disease, lung cancer, and esophageal and proximal gastric cancer may still receive either a high dose of radiation to a small part of the heart or a lower dose to the whole heart [7–11]. In addition, although there is increasing use of concomitant therapies, the extent to which these therapies affect radiotherapy side effects such as RIHD is largely unknown.

Manifestations of RIHD include accelerated atherosclerosis, pericardial and myocardial fibrosis, conduction abnormalities, and injury to cardiac valves [4, 12]. The disease is progressive and both incidence and severity increase with a higher radiation dose volume, younger age at the time of radiotherapy, a greater time elapsed since treatment, and concomitant use of cardiotoxic chemotherapeutic agents such as anthracyclines. Although RIHD is widely acknowledged as an impediment to quality of life for certain long-term cancer survivors, from a clinical perspective the only current way to reduce RIHD is through efforts to improve radiotherapy treatment planning, as other methods to prevent or reverse RIHD are not yet available. Hence, pre-clinical studies seek to unravel basic mechanisms of RIHD, with the ultimate goal to identify potential targets for intervention.

2. Pre-Clinical Models of Radiation-Induced Heart Disease

Pre-clinical animal models have long been used to study RIHD [13–18]. While transgenic mouse models are being used in investigations of radiation-accelerated atherosclerosis

[19, 20], wild type rodents are usually not atherosclerosis prone. Hence, studies that use rodents to investigate radiation-induced coronary artery disease are limited in number [21, 22]. On the other hand, many laboratory animals, including rodent, have been used successfully as models of radiation-induced cardiomyopathy [16, 23–27]. Common doses used in these pre-clinical models of localized heart irradiation are either a single dose between 5 Gy and 25 Gy, or fractionated schedules of, for instance, 5 daily fractions of 9 Gy. Some of the histopathological changes in pre-clinical models, such as myocardial degeneration and fibrosis, are also commonly described in human cases of RIHD, mainly after exposure to doses of ~30 Gy and above [3, 4, 28–30]. Although clinical and pre-clinical data on the cardiovascular effects of lower radiation doses are growing [11, 31], the focus of this review will be on myocardial injury and cardiac function changes after exposure to higher doses of radiation. Table 1 summarizes some of the main pre-clinical studies reviewed.

3. Vascular Injury and Endothelial Dysfunction

Previous paper indicate the important role of vascular injury and endothelial dysfunction (loss of thromboresistance and increased expression of adhesion molecules and cytokines) in the pathogenesis of normal tissue radiation injury [42, 43]. Endothelial dysfunction may contribute to profibrotic and proinflammatory environments, which are common aspects of normal tissue radiation injury [42, 44]. Although the role of endothelial dysfunction in RIHD has not been studied in detail, experimental RIHD is known to be associated with reduced myocardial capillary density [32, 33], focal loss of endothelial alkaline phosphatase [14, 34], and increased expression of von Willebrand factor [35]. Hence, microvascular injury and the resulting local ischemic injury are considered to be some of the underlying mechanisms of RIHD.

Radiation-induced vascular injury and endothelial dysfunction are mediated in part by Transforming Growth Factor- β (TGF- β) [45, 46], a pluripotent growth factor that is part of many normal tissue radiation responses [47–49]. Previous studies have shown cardiac upregulation of TGF- β in rat models of RIHD after localized heart irradiation with 20 Gy or 5 fractions of 9 Gy [36–38]. A TGF- β -inducing compound was used to investigate the role of TGF- β in RIHD in the rat. Cardiac radiation fibrosis was more severe in animals that had been administered the TGF- β -inducing compound during the 6-month followup time after irradiation (unpublished data). Pre-clinical studies involving TGF- β receptor inhibition are being undertaken.

4. Mast Cells

Mast cells, cells that belong to the hematopoietic myeloid lineage, reside in many organs and tissues including the heart. Although best known for their role in hypersensitivity reactions, mast cells are also intimately involved in wound healing and tissue remodeling [50–52]. Mast cells store

and release a wide range of cellular mediators, both via degranulation and via constitutive pathways that do not involve degranulation [53]. Increased mast cell numbers are commonly found in coronary atherosclerosis, myocardial fibrosis [54, 55], and also in animal models of RIHD [40, 56], where mast cell numbers correlate with myocardial radiation injury.

The development and maturation of mast cells depend on the c-kit receptor, which is specific for stem cell factor. Several mast cell deficient animal models, based on a mutation in the c-kit receptor or stem cell factor, are available [57–59]. Our laboratories have made use of a rat model that is homozygous for a 12-base deletion in the c-kit receptor gene [60, 61]. Both mast cell-deficient rats and their mast cell-competent wild type litter mates were exposed to localized heart irradiation with a single dose of 18 Gy. Although mast cell-deficiency was associated with reduced radiation-induced myocardial inflammation and degeneration, other manifestations of cardiac radiation injury such as myocardial fibrosis and *ex vivo* measures of myocardial stiffness were exacerbated in the absence of mast cells [41]. These studies suggest that mast cells, in contrast to what had been the prevailing assumption but similar to what has been found in some other cardiac disease models [62, 63], play a predominantly protective role in RIHD.

5. Mast Cell Interactions

Mast cells interact with many cellular and molecular systems in the heart. Mast cell-derived proteinases, for instance, have been shown to contribute to both the formation and degradation of endothelin-1 (ET-1) [64–68]. ET-1 is a 21-amino acid peptide that was first discovered as a potent vasoconstrictor but also has proinflammatory and pro-fibrotic properties [69, 70]. The role of ET-1 in cardiovascular pathology has been studied extensively [71, 72]. Both ET-1 receptors, ET_A and ET_B are expressed by a wide variety of cell types in the heart [70, 73]. Short-term upregulation of ET-1 and its receptors may serve as a mechanism to maintain cardiac function in certain cardiovascular diseases [74, 75]. Long-term up-regulation of the endothelin system, on the other hand, may have detrimental effects due to the vasopressor, prohypertrophic, and pro-fibrotic properties of ET-1 [73, 76].

Mast cells express the receptor ET_A, which upon activation by ET-1 induces mast cell degranulation [77], a pathway by which ET-1 may enhance the activity of matrix metalloproteinases (MMPs) in the heart [78, 79]. Dual inhibition of ET_A and ET_B prevented mast cell degranulation and the associated increase in cardiac MMP levels, interstitial collagen degradation, and ventricular dilatation in a rat model of chronic volume overload [80]. On the other hand, in a preliminary study of a rat model of RIHD, dual inhibition of ET_A and ET_B did not alter radiation-induced functional or structural cardiac changes [81]. Moreover, vascular injury seemed aggravated by selective ET_A inhibition in a rat model of localized intestine irradiation [82]. Dosing of receptor antagonists and opposing cardiovascular effects of

TABLE 1: Summary of pre-clinical studies into basic mechanisms of RIHD.

Main Observation or Study Outcome	References
Reduced myocardial capillary density, focal loss of endothelial alkaline phosphatase, and increased expression of von Willebrand factor indicate vascular injury in rat models of RIHD.	[14, 32–35]
Coronary artery disease has been observed after localized heart irradiation in hypertensive rats or rats on a high-fat diet.	[21, 22]
Increased myocardial levels of TGF- β 1, Ang II, and aldosterone have been found after localized heart irradiation in rats.	[36–39]
ACE inhibitor captopril reduced myocardial fibrosis and prevented left ventricular capillary density loss after localized heart irradiation in rats.	[40]
Mast cell-deficient rats showed reduced radiation-induced myocardial inflammation and degeneration, but increased myocardial fibrosis when compared to mast cell-competent rats.	[41]

the ET_A and ET_B receptors [83, 84] warrant further studies to clarify the role of ET-1 and its two receptors in RIHD.

Mast cells are one of the main cell types involved in neuroimmune interactions [85]. They are found in close proximity to nerve terminals or axons in many organs, including the heart [86, 87], and interact with nerves on the molecular level in many ways [85, 88, 89]. Mast cells express α - and β -adrenergic receptors [90, 91]. In normal rat myocardium, β -blockade is associated with increased mast cell degranulation and decreased collagen deposition [92]. Some sensory neuropeptides such as calcitonin gene-related peptide (CGRP), substance P, and neuropeptide Y are able to induce or enhance mast cell degranulation [93–100] while others have been shown to inhibit mast cell degranulation [101, 102]. Mast cells, in turn, affect neuronal growth and function by producing nerve growth factor [103] and by activating proteinase-activated receptor-2 on the surface of neurons [104, 105]. Cardiac sensory nerves play a protective role in the heart via the release of nitric oxide and CGRP [106, 107]. For instance, CGRP plays a protective role in myocardial injury such as from ischemia reperfusion and the cardiotoxic chemotherapeutic agent doxorubicin [108, 109]. CGRP is a potent vasodilator but also has beneficial effects in the heart by local downregulation of tumor necrosis factor- α (TNF- α) and upregulation of insulin-like growth factor-1 (IGF-1) [110, 111]. Interestingly, both downregulation of TNF- α and upregulation of IGF-1 are associated with reduced normal tissue radiation injury [112, 113]. In line with this evidence, CGRP has been shown to protect in a rat model of radiation enteropathy [114]. Its role in RIHD has not yet been studied extensively.

6. The Renin-Angiotensin System

The role of the renin angiotensin system (RAS) in normal tissue radiation injury has been well defined [115, 116]. Inhibitors of angiotensin-converting enzyme (ACE) and antagonists of angiotensin type 1 receptors reduce injury in animal models of localized kidney, lung, and brain irradiation [117–119]. Although the role of RAS in RIHD is less well defined, RAS mediators may be upregulated in the heart after irradiation [39]. However, while the ACE inhibitor captopril reduced radiation injury in kidney, lung, and skin of rats [119–121], captopril did not prevent cardiac

function loss after localized heart irradiation with 20 Gy in a rat model. Captopril, on the other hand, did reduce myocardial fibrosis and prevented left ventricular capillary density loss after local heart irradiation. It is not known whether these effects were due to properties of captopril other than its inhibition of ACE [40].

Inhibition of ACE is considered to be cardioprotective in part by suppressing the breakdown of bradykinin by ACE [122]. Bradykinin, a small peptide hormone that is sometimes considered to aggravate cardiac disease with a significant inflammatory component such as myocardial infarction [123], is also known to mediate cardioprotection via induction of nitric oxide and prostacyclin [124–126]. Bradykinin is formed in the kallikrein-kinin system by proteolytic cleavage of both high- and low-molecular weight kininogen by kallikrein enzymes, but also by the mast cell-derived enzyme tryptase [127, 128]. Interestingly, the mast cell proteinases chymases are one of the main converters of angiotensin I into angiotensin II [129]. Mast cells seem to hereby provide a particularly large contribution to local extravascular generation of Ang II [130]. The roles of RAS and bradykinin in cardiac radiation injury and the potential influence of mast cells herein need further investigation.

7. Conclusions

Radiotherapy planning has undergone many improvements over the last decades, leading to improved targeting and reduced normal tissue radiation exposure. Nonetheless, some patients with Hodgkin's Disease, lung cancer, and esophageal and proximal gastric cancer may still receive either a high dose of radiation to a small part of the heart or a lower dose to the whole heart, which may lead to late manifestations of RIHD. Some of the basic mechanisms of RIHD have begun to emerge from recent pre-clinical studies and include the involvement of vascular injury, mast cells, and the RAS. Future studies will elucidate the significance of these mechanisms for clinical RIHD and their usefulness as targets for intervention in RIHD.

Conflicts of Interest

The authors have no potential conflicts of interest.

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