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Vascular Injury Following Whole Thoracic X-ray Irradiation in the Rat

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Abstract

Purpose

To study vascular injury following whole thoracic irradiation with single sublethal doses of X-rays in the rat and to develop markers that might predict severity of injury.

Materials and Methods

Rats after 5 or 10 Gy thorax only irradiation and age-matched controls were studied at 3 days, 2 weeks, 1, 2, 5 and 12 months. Several pulmonary

vascular parameters were evaluated, including hemodynamics, vessel density, total lung angiotensin converting enzyme activity and right ventricular hypertrophy.

Results

By 1 month, rats in the 10 Gy group had pulmonary vascular dropout, right ventricular hypertrophy, increased pulmonary vascular resistance, increased dry lung weights, and decreases in total lung angiotensin converting enzyme activity as well as pulmonary artery distensibility. On the other hand irradiation with 5 Gy resulted only in a modest increase in right ventricular weight and a reduction in lung angiotensin converting enzyme activity.

Conclusions

In a previous investigation employing the same model we observed that there was recovery from radiation induced attenuation of pulmonary vascular reactivity. In this study we report that there is deterioration in several vascular parameters up to 1 year following exposure to 10 Gy suggesting sustained remodeling of the pulmonary vasculature. Our data support clinically relevant injuries which appear in a time and dose related manner after exposure to relatively low doses of radiation.

Keywords: radiation, vascular dropout, distensibility, vascular resistance

Introduction

Lung injury following exposure to high dose radiation during cancer treatment is well described. While radiation injuries to the kidneys or hematopoietic system may occur at lower doses than pulmonary injuries, better supportive therapies for renal and blood cell disorders are available than for lung failure, potentially leaving impaired pulmonary function as the survival limiting factor. Furthermore, the response of the lung to exposure in the 1–10 Gy range is not well investigated ([1](#)). This dose range assumes importance in the face of the growing threat of nuclear accidents and radiological terrorism, with exposure possible, to survivable doses of radiation. Any attempt to adequately respond to such incidents will require the capability to accurately predict and interpret the expression of radiation injury in different organ systems.

Radiation injury is believed to result from a combination of the direct effects of radiation, the inflammatory process and abnormal wound healing. Several mediators including reactive oxygen and nitrogen species, cytokines, growth factors and intermediates of the renin-angiotensin system are involved in this complex process (2). Injury of blood vessels is believed to be a primary determinant of the resultant effects of radiation in a variety of organs including the lungs (3). Clinical studies conducted in the early 1970's examined the relationship between the relative pulmonary blood flow and irradiation. The blood flow was found to decrease in regions of the lungs irradiated with 40 Gy although the mechanism was not clear (4). Recent studies in rats demonstrate changes in lung perfusion occurring as early as 3 days after hemithoracic irradiation, with 28 Gy of X-rays, which initiate the development of hypoxia and chronic oxidative stress that perpetuate injury (5). We have confirmed that reactivity of pulmonary arteries is attenuated following injury with 10 Gy to the whole thorax. The drop in reactivity coincided with other structural and functional indices of pulmonary dysfunction (6). In this study we measured several parameters to more completely characterize pulmonary vascular status in rats after single whole thoracic irradiation using sublethal doses. Hemodynamic and structural alterations in pulmonary vasculature are also likely to have an impact on cardiac function. McChesney et al. demonstrated that structural and functional cardiac damage as well as pulmonary hypertension were more pronounced in dogs following 12 Gy whole thoracic irradiation, compared to irradiation of limited volumes of lungs and the heart (7, 8). In addition to being an important factor in the development, progression and resolution of radiation induced cardiopulmonary dysfunction, remodeling in the pulmonary vascular bed could also be a target for therapeutic intervention following radiation exposure.

A suitable animal model with well-defined features is needed to further understand processes that underlie radiation injury with sublethal doses at tissue, and whole animal level. Such knowledge will be crucial in accurately evaluating the efficacy of radioprotectors and therapeutic agents as well as monitoring individuals with survivable radiation injury. We have developed a rat model of radiation induced lung injury (6) and sequentially assessed the changes in cardiopulmonary physiology and morphology that result from whole thoracic irradiation with single doses of 5 or 10 Gy of X-rays.

Methods

Animals

All the studies were done under approval of the Medical College of Wisconsin and Zablocki VA Medical Center IACUC review boards and in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Injury model

Unanesthetized female WAG/Rij/MCW rats (110–170 gm, N=144) were placed in a plexiglas holding jig and given 5 or 10 Gy of radiation limited to the thorax. A Pantak HF320 orthovoltage system (Therapax, Danbury, CT, USA) was used, with a 300 kVp beam, a halfvalue layer of 1.4 mm Cu, and a midline dose-rate of 1.615 Gy/min as previously described (6). The absolute dose was measured using a calibrated Farmer-type ionization chamber. The radiation dose was delivered by two equally-weighted lateral beams in order to improve the dose uniformity. An 8 × 7.5 cm² collimator was used to define a radiation field that encompassed the whole thorax; the whole lung is in the field, plus the heart and a small amount of the liver.

Irradiated rats and their age-matched controls were studied at 3 days, 2 weeks, and 1, 2, 5, and 12-months after irradiation.

Lung preparation, imaging and analysis

Lung preparation, imaging methods and analysis follow those previously described (9). Rats were anesthetized with sodium pentobarbital (40mg/kg), a midline sternotomy performed, the animal was heparinized and blood sampled via right ventricle, the trachea and pulmonary artery were cannulated, and the heart excised and lungs removed. The lungs were hung by the cannulae, ventilated, rinsed of blood, and perfused with a 5% BSA physiological saline solution (Equitech-Bio Inc, Kerrville, Texas, USA).

Pulmonary artery pressure was measured at 30, 20, 15, 10, 5 ml/min, the flow was then stopped and closing pressure recorded.

Flow rates were normalized by the rat body weight, and data fit to a simple pulmonary vascular distensibility model ([10](#)) to determine pulmonary vascular resistance at 100 ml/min/kg. The perfusate in the arteries was replaced with 1-bromo-perfluoro-octane (Crescent Chemical Co. Inc, Islandia NY, USA) for X-ray contrast, intravascular pressure set to 30, 21, 12, and 5 mmHg and microfocal angiographic imaging (including volumetric CT) performed at each pressure.

Arterial distensibility was measured using previously published methods ([9](#)). Briefly, isotropic CT volumes were used to map and measure the main pulmonary trunk at each intravascular pressure. The data were fit, using an unconstrained multivariable non-linear optimization function 'fminunc' in the Matlab optimization toolbox (MathWorks, Natick, MA), to a morphometric model of the pulmonary arterial tree, which incorporates a well-accepted model of pulmonary arterial distensibility. To quantify microvascular density, high-magnification planar angiograms of the right, lower lung lobe, imaged at an intravascular pressure of 30 mmHg, were normalized to their respective flood field. An operator then selected a region of interest (ROI, 200×100 pixels) along the base of the lung. A histogram of the data within the ROI was calculated and the gray-scale difference between that at the 90th percentile and the mode was subtracted from the value at the mode to determine a threshold. The threshold was then used to transform the ROI into binary data, pixels below the threshold were categorized as 'vessel' and above the threshold as 'background'. The number of pixels segmented as vessel was divided by the total number of pixels in the ROI to determine an index of microvascular density index.

Right ventricular weight and hematocrit

Following excision of the heart, the right ventricle was dissected and right ventricular hypertrophy was assessed as the ratio of the weight of the **right ventricle** to the weight of the **left ventricle plus septum** (RV/LV+S). Blood collected from the heart was centrifuged at 30,000 rpm for 2 minutes and the ratio of packed cells to plasma used as the measure of hematocrit.

Dry lung weight

After imaging, the trachea and main pulmonary artery were trimmed off and the lungs placed in an oven for at least 2 days before weighing. Dry lung weights were normalized to body weight to provide an index to access changes in total lung composition.

Total lung Angiotensin Converting Enzyme (ACE) activity

N-[3-(2-furyl)-acryloyl] – L-phenyl-alanylglycylglycine [FAPGG] (Sigma-Aldrich, Milwaukee, USA) was perfused through the isolated lung and lung ACE binding activity measured as the percent conversion of FAPGG to FAP in the effluent, measured spectrophotometrically ([11](#)). ACE activity is presented as mean surface area product (MSAP).

Statistical analysis

Data are calculated and presented as mean $\pm$ standard error of the mean. All statistical comparisons were performed in SigmaStat 3.0 (SPSS, Chicago, IL) using an unpaired t-test ($P \leq 0.02$). If the normality test failed a Mann-Whitney Rank Sum test was run to confirm significance. All comparisons were made between age-matched unirradiated controls and irradiated animals.

Results

Body weight

Body weight was observed to vary with the dose. No reduction in body weight was observed in rats irradiated with 5 Gy compared to age matched controls at any end-points. However, rats irradiated with 10 Gy had a significant decrease in body weight compared to age-matched controls at 2 and 12 months ([figure 1](#)).

Fig. 1 Body weight of rats given 5 or 10 Gy X-rays compared to age-matched controls measured at the indicated time points (mean $\pm$ sem). There was a significant reduction in body weight in rats irradiated with 10 Gy compared to age-matched controls at 2 and 12 months (*/ τ $p \leq 0.02$ vs age-matched controls).

Hematocrit

Hematocrit did not vary following the two doses employed and no significant differences were recorded in rats irradiated with either 5 Gy or 10 Gy compared to age-matched controls (data not shown).

Total lung ACE activity

Total ACE activity measured in isolated, perfused lungs was significantly reduced at 1, 2, 5 and 12 months following 10 Gy, and at 12 months only, after 5 Gy as shown in [figure 2](#).

Fig. 2 Total lung angiotensin converting enzyme activity was measured by means of a spectrophotometric assay using FAPGG as a substrate. The values represent total ACE activity in the lungs of rats irradiated with 5 or 10 Gy at the 6 different time points up to 1 year (mean $\pm$ sem). Significant reduction in ACE activity was observed 2, 5 and 12 months after 10 Gy compared to age-matched controls and at 12 months after 5 Gy. ($p \leq 0.02$ vs age-matched controls).

Pulmonary vascular resistance

Pulmonary vascular resistance (PVR) was measured in isolated-perfused, whole lungs to obtain results independent of hematocrit. PVR is reported at a constant flow rate of 100 ml/min/kg and was found to be significantly elevated at 1, 2 and 5 months following irradiation with 10 Gy (figure 3). By 12 months however, the increase in vascular resistance resolved. No significant differences were noted between the 5 Gy rats and their age-matched controls at any end points.

Fig. 3 Pulmonary Vascular Resistance values independent of hematocrit and blood volume in rats irradiated with 5 or 10 Gy X-rays compared to age matched controls at the indicated time points (mean $\pm$ sem). Pulmonary vascular resistance that developed at a constant flow rate of 100 ml/min/kg was found to be significantly elevated at 1, 2 and 5 months following 10 Gy compared to age-matched controls, with resolution by 12 months. No significant differences were noted between the rats irradiated with 5 Gy and their age-matched controls at any of the time points (*/ ψ / θ $p \leq 0.02$ vs age-matched controls).

Right ventricular hypertrophy

Whole thoracic irradiation resulted in observable right ventricular hypertrophy (RVH). Following irradiation with 5 Gy, there was evidence of RVH at 5 months, while exposure to 10 Gy resulted in RVH as early as 2 months. Interestingly, there was resolution of RVH in both irradiated cohorts, with no difference between age-matched controls at 12 months following 5 Gy or at 5 months following 10 Gy ([figure 4](#)).

Fig. 4 The ratio of the weight of the right ventricle versus the sum of the left ventricle and septum (RV/LV+S) calculated in rats irradiated with 5 or 10 Gy of X-rays compared to age matched controls at the time points indicated (mean $\pm$ sem). There was a modest but significant increase in this ratio in rats irradiated with 5 Gy compared to age matched controls at 5 months, with resolution by 12 months. A significant elevation was also noted at 2 months after 10 Gy of X-rays compared to age matched controls with resolution by 5 months (*/# $p \leq 0.02$ vs age-matched controls).

Dry lung weight

There was significant elevation in dry lung weight index at 1, 2, 5 and 12 months after 10 Gy, with marked increases noted at 1 and 2 months. This increased dry weight is reflective of the underlying vascular remodeling. No increase was found following injury with 5 Gy ([figure 5](#)).

Fig. 6 Low-magnification (top) and high-magnification (bottom) pulmonary arterial angiograms in lungs isolated from a rat 2 months after a dose of 10 Gy (right) and an age-matched control rat (left) in which intravascular pressure was at 30 mmHg and airway pressure at 6 mmHg. In low-magnification images, the pulmonary artery cannula is 1.67 mm in diameter. In the high-magnification images, the scale bar (lower-left image) indicates approximately 500 microns.

Fig. 7 Pulmonary microvascular density measured in rats after 10 Gy compared to age-matched controls at the indicated time points (mean $\pm$ sem). Vascular density was significantly decreased at 1,2,5 and 12 months following irradiation with 10 Gy ($^{\wedge}/^{\wedge}/^{\wedge}/^{\wedge}$ $p \leq 0.02$ vs age-matched controls).

Fig. 8 Pulmonary arterial distensibility at 2 wks, 1, 2, 5 and 12 months following 10 Gy of X-rays compared to age-matched controls (mean $\pm$ sem). Vascular diameters were obtained from corresponding angiograms and the distensibility (change in diameter for unit changes in pressure) was computed for each group. A significant reduction in distensibility was observed at 2 months and persisted at 5 and 12 months following irradiation with 10 Gy (*/^/\$ $p \leq 0.02$ vs age-matched controls).

Discussion

Large dose radiation injury from fractionated radiation exposure of the thorax has been investigated due to the use of therapeutic radiation for lung tumors or lymphoma. The consequences of single exposure to lower doses has only recently received attention because of the threat of radiological terrorism. In addition, pulmonary fibrosis is a well-recognized complication of total body irradiation (TBI) including the lungs developing months to years after TBI with more modest doses (10–12 Gy total) for malignancies (12). In the context of radiological terrorism, however, pulmonary fibrosis is less relevant than pneumonitis, which is likely to be the major cause of death at doses where the GI and hematopoietic syndromes are potentially survivable.

We examined cardiopulmonary structure and physiology in rats given single doses of 5 or 10 Gy to the whole thorax, with the aim of developing a better description of the natural history of this injury, including the development of markers that might predict severity of injury after survivable doses of radiation. In a previous study we had noted that there was recovery of structural and functional pulmonary dysfunction following thoracic irradiation with 10 Gy. In this study we show that there is reversible deterioration in numerous parameters following 10 Gy, with increases in PVR and right ventricular weight commencing at around 1–2 month post exposure and recovering by 12 months. On the other hand, reduction in pulmonary arterial distensibility, vessel density and total lung ACE activity and the elevation in dry lung weight index persisted up to 12 months after irradiation. A dose of 5 Gy resulted in minimal dysfunction with a transient, and modest, increase in right ventricular weight at 5 months following irradiation, and a reduction in total lung ACE activity at 12 months ([Table 1](#)).

Endpoints	Dose	Times Post Irradiation					
		3 Days	2 Weeks	1 Month	2 Months	5 Months	1 Year
Body Weight	5 Gy	±	±	±	±	±	±
	10 Gy	±	±	±	↓	±	↓
Total Lung ACE activity	5 Gy	±	±	±	±	±	↓
	10 Gy	±	±	↓	↓	↓	↓
Pulmonary Vascular Resistance	5 Gy	±	±	±	±	±	±
	10 Gy	±	±	↑	↑	↑	±
Right Ventricular Hypertrophy	5 Gy	±	±	±	±	↑	±
	10 Gy	±	±	±	↑	±	±
Dry Lung Weight	5 Gy	±	±	±	±	±	±
	10 Gy	±	±	↑	↑	↑	↑
Vessel Density	5 Gy	±	±	±	±	±	±
	10 Gy	±	±	↓	↓	↓	↓
Arterial Distensibility	5 Gy	±	±	±	±	±	±
	10 Gy	±	±	±	↓	↓	↓

Table 1 Summary of findings throughout study duration.

Table summarizing the results of the assays conducted in rats irradiated with 5 and 10 Gy at all the time points studied. '±' indicates no significant change in the index compared to age-matched controls. '↓' indicates a significant reduction compared to age-matched controls and '↑' represents a significant elevation in the index compared to age-matched controls.

It has been speculated that changes in the vasculature and interstitial connective tissue play a vital role in the production of chronic lesions after irradiation, irrespective of radiosensitivity or regenerative capacity of the parenchyma. In addition, the degree to which the organ or tissue can return to structural and functional integrity, is also determined, to a large extent, by the capacity of the vasculature to regenerate ([13](#)).

Vujaskovic et al. demonstrated that there is a significant reduction in blood flow in the irradiated lung 4 weeks after hemithoracic irradiation with 28 Gy ([5](#)). Several other investigators have demonstrated a dose dependant reduction in microvascular surface area and blood flow in irradiated tissue ([14,15](#)) with recovery depending on numerous variables including dose, dose rate, and the type of tissue involved. The processes underlying this vascular remodeling are known to include smooth muscle cell growth and migration, endothelial cell dysfunction, inflammation and both extracellular matrix synthesis and degradation ([16,17](#)). In the lungs, this injury is manifest as abnormalities in hemodynamics and results in an increase in pulmonary vascular resistance. We have observed significant impairments in several pulmonary vascular parameters following a dose of 10 Gy limited to the thorax. Pulmonary vascular resistance increased and distensibility started to decrease around 4–8 wks after irradiation ([Figure 3](#) and [and8](#)).[8](#)). This coincided with the occurrence of pulmonary vascular dropout ([Figure 6](#) and [and7](#)).[7](#)), indicating significant remodeling, with single exposure of the thorax to 10 Gy. Interestingly, after 10 Gy, the increase in PVR resolved by 12 months while the reduction in vessel density as well as distensibility persisted. Distensibility is known to result from structural alterations in the vasculature including increased collagen deposition as well as extracellular matrix modulation and parenchymal changes ([18](#)). These changes are reported in pulmonary vascular diseases related to valvular heart disorders, congenital heart disease and conditions

decreasing ventricular compliance ([19](#)). In rats, hypoxia has been shown to cause a doubling in thickness of the medial and adventitial coats of the hilar muscular pulmonary artery. With recovery, although normal thickness was regained, there was an increase in collagen fiber deposition which resulted in a persistent reduction in distensibility in these vessels ([20](#)). We have observed that vascular dropout precedes the reduction in distensibility and it is possible that this increase in arterial stiffness is in part a reflection of the remodeling that follows suboptimal perfusion. Decreased distensibility in the setting of radiation injury could thus be a sign of a lasting impairment in the ability to adapt to added injury or stress such as inflammation or infection. Increased ventricular volume or pressure overload is known to result in compensatory cardiac restructuring ([21](#)). Even though exposure to 5 Gy did not result in detectable pulmonary hemodynamic alterations, there was evidence of modest right ventricular hypertrophy at 5 months which resolved by 12 months post injury, suggesting a reversible increase in the workload of the right heart. On the other hand, right ventricular hypertrophy following exposure to 10 Gy ([figure 4](#)) paralleled the increase in pulmonary vascular resistance and vessel dropout indirectly confirming a difference, not only in the extent of pulmonary vascular remodeling resulting from 5 Gy compared to 10 Gy but also a considerable difference in temporal evolution.

Following injury with 10 Gy, the dry lung weight index was found to be increased ([Figure 5](#)). Radiation pneumonitis in rats is characterized by early inflammatory cellular infiltration and fibrin deposition especially in the perivascular and peribronchial region. This is followed by a phase of chronic inflammation and fibrocellular proliferation that develops months to years after irradiation ([22](#)). Although non specific, an elevation in normalized dry lung weight suggests an increase in lung cellularity and possibly the presence of interstitial fibrosis, findings which were prominent on histological analysis of lung samples in our other study employing the same model ([6](#)).

ACE is known to localize to the luminal surface of the lung's vascular endothelium. ACE activity is a well-documented index of endothelial functional status and has been used to monitor lung injury resulting from a variety of insults. Ward and his colleagues

demonstrated a reduction in ACE activity to 20 % of control levels, starting at 1 month post exposure, in a rat model of hemithoracic irradiation with 25 Gy (23). This association between endothelial enzymatic activity and subsequent radiation pneumotoxicity was evident in a study with mice where strains prone to radiation induced pulmonary fibrosis had significantly lower ACE activity compared to strains resistant to fibrosis (24). We confirm a reduction in total lung ACE activity commencing at 1 month and persisting up to 12 months following 10 Gy to the whole thorax of the rat, but only at 12 months after 5 Gy (Figure 2). There is substantive evidence that ACE and ACE-generated proteins modulate the vascular remodeling that accompanies various pathological processes including radiation injury (16). In addition, ACE inhibitors like captopril have been shown to have a protective effect on radiation induced pulmonary injury (25). An explanation for these disparate findings is that total lung ACE activity does not reflect local pulmonary vascular ACE expression. In a study conducted in chronically hypoxic rats, total lung ACE activity and ACE activity of alveolar capillary endothelium were reduced, but in situ hybridization studies showed an increased signal for ACE mRNA in newly muscularized pulmonary arteries at the level of the alveolar ducts and walls. The increased signal involved the entire vessel wall rather than the endothelium alone (26). These findings raise the interesting possibility that radiation results in similar changes, with ACE expression being increased in the walls of small pulmonary arteries despite a generalized reduction in total lung ACE activity. The pattern of ACE expression along the walls of pulmonary vessels following thoracic irradiation warrants further exploration.

These data are essential for 2 purposes. First, they are needed to estimate the risk and presentation of injury as well as optimal screening procedures for patients with possible exposure. Second, to test any mitigating or therapeutic interventions, the natural history of a single dose of radiation to the thorax must be known.

Further studies are needed to determine if the deterioration in pulmonary arterial distensibility and vessel density following whole thoracic irradiation with 5 or 10 Gy progress beyond 1 year after irradiation and, whether recovery in the other parameters is confounded by additional injury such as infection.

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Footnotes

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