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Summary

We describe a rat model of post-irradiation (p.i.) pulmonary vascular injury resultant from low-dose radiation exposure. The model was developed to investigate radiation exposure injuries expected in casualties resulting from a radiological attack. Rats were challenged with a single dose of 5 or 10 Gy of radiation or a sham irradiation procedure. Animals were studied at time points from 3-days to 12-months. We measured various physiological parameters and used a novel technique of X-ray microfocal angiographic imaging to investigate pulmonary arterial morphology. With our analysis, exposure to 5 Gy had no measurable effect on pulmonary arterial structure/function, whereas 10 Gy resulted in a number of significant physiological abnormalities including increased vascular resistance, a decrease in total lung ACE activity, and vessel dropout.

Introduction

We hypothesize that pulmonary injury will manifest in survivors of radiological attacks after they have been treated for more acute injury to the bone marrow, gastrointestinal tract and kidney. In our model of low-dose injury, we use radiation limited to the thorax to reduce the confounding hemopoietic, gastrointestinal and renal effects in order to isolate lung pathobiology. The long-term goals of this work, in response to the cited need[1, 2], are to find treatments and/or mitigators that will improve lung health and function[3]. In order to accomplish our goals, we have worked on methods to quantify

pneumopathy caused by radiation exposure levels likely to be present in casualties associated with a radiological dispersal device, or lower exposures compared to existing radiation therapy models[4-6].

Although the pathogenesis of radiation-induced pneumopathy remains controversial, some groups have focused on radiation-induced endothelial cell death or dysfunction[7] since changes in angiotensin converting enzyme, prostaglandins, thromboxane, endothelin, plasminogen activator, and platelet activating factor have been found in models of lung irradiation. Others believe type II pneumocytes are the critical target and that surfactant released by the damaged type II cells triggers the radiation injury response[8]. A more likely scenario is that the radiation response includes some contribution from both mechanisms[9]. Our studies examined isolated perfused lung preparations to measure metabolic, hemodynamic, and structural changes that result at different p.i. time points. At the 5 and 10 Gy dosages used in our present models, there appears to be an early response, which includes phased changes in and recoverable damage to the vasculature, and a late response resulting in interstitial lung disease and fibrosis.

Materials and Methods

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. Unanesthetized female WAG/R$_{ij}$/MCW rats (110-170 gm, N=144) were placed in a plexiglass holding jig and exposed to 0, 5, or 10 Gy of radiation limited to the thorax by lead shielding. The X-ray field was from posterior to anterior at a dose rate of ~2 Gy/minute. Exposed animals and their age-matched controls were studied at time points of 3-days, 2-weeks, and 1, 2, 5, and 12-months(m) after irradiation or similar sham procedure. Lung preparation, imaging methods and analysis follow those previously described[10]. Briefly, animals 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 cunnulated, and the heart and lungs removed. The left and right heart were weighed to assess hypertrophy. The lungs were hung by the cannulae, ventilated, rinsed of blood, and perfused with a 5% BSA physiological saline solution. Angiotensin converting enzyme activity[11] and pulmonary vascular resistance were measured in the isolated perfused lungs. Then the perfusate in the arteries was replaced with perfluorooctyl bromide for X-ray contrast and high-resolution angiograms of the lungs were acquired and used to analyze morphological changes in the vessels. Lastly, the lungs were placed in a drying oven for several days then weighed and normalized to body weight to access changes in total lung composition. All statistical comparisons were performed using a t-test (Pd"0.02) in SigmaStat 3.0 (SPSS, Chicago, IL).

Results

There was a dose dependent response to irradiation, with the 5Gy groups showing little measurable changes (no significant differences) whereas the 10Gy groups had significant differences, in a number of parameters, that peak around 2-ms p.i., compared to their respective age matched controls. Hematocrit measured from blood sampled in the animals at time of sacrifice was not significantly different in any of the groups (data not presented). Total lung ACE activity was significantly reduced in the lungs of 10Gy rats beginning 1-m p.i., see Figure 1. A qualitative analysis of angiograms of the lungs indicated that micro-vascular density was reduced beginning at 1-m p.i. This rarefaction or vessel dropout appears to peak at 2-m p.i. and then return to near control status by 12-m p.i., see Figure 2. The dry lung weight, which was normalized to body weight, was significantly increased in 10Gy rats beginning at 1-m p.i., see Figure 3.

Conclusion

Thoracic irradiation of 5 and 10Gy had a dose dependent but delayed impact on the normal structure and function of the pulmonary vasculature and related cardiovascular parameters, most of which reached significance 1-to-2-m p.i. in the 10Gy group. Irradiation with 5Gy had no significant effect, in contrast 10Gy irradiation resulted in decreased ACE activity after 1-m, and
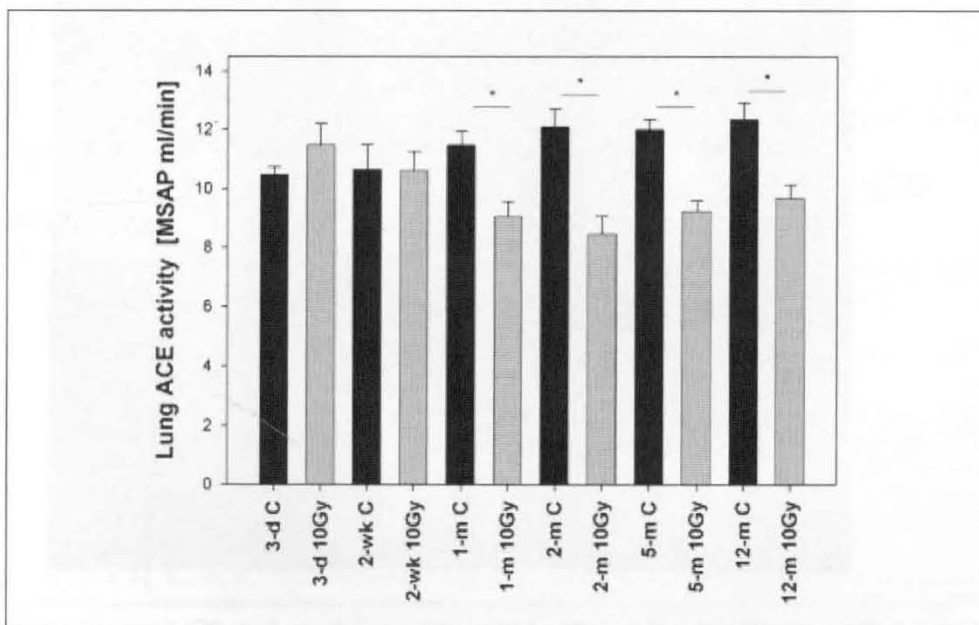

Figure 1. Total lung ACE activity presented as mean surface area product (MSAP) measured in lungs of control and irradiated rat at 3-d, 2-wk, 1-m, 2-m, 5-m, and 12-m.

increased normalized dry lung weight after 1-m p.i.. There was a decrease in micro-vascular (< 200μm) density after 1-m p.i, which peaked at the 2-m p.i., after which some of the injuries began to resolve. Although our experiments did not directly test for measures of fibrosis such as total lung compliance,
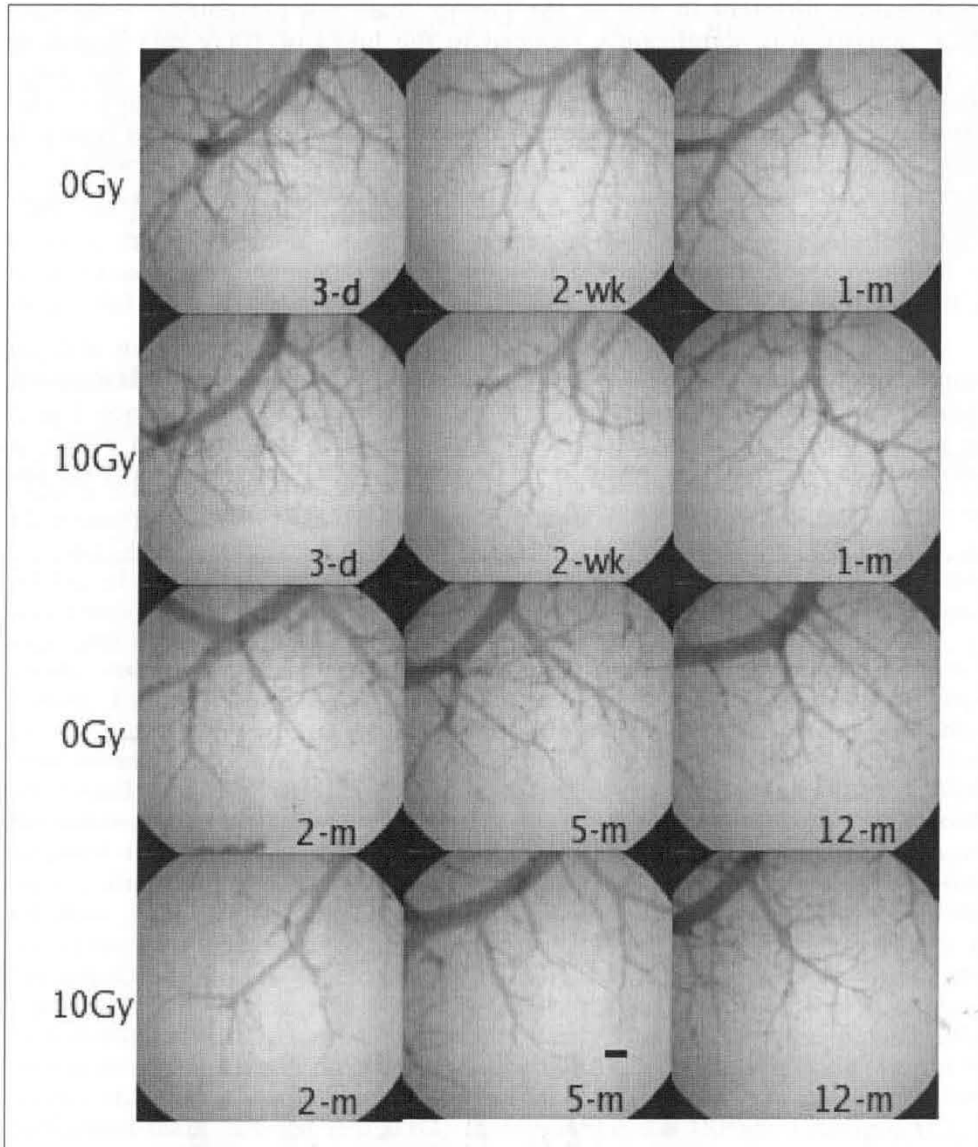

Figure 2. High-magnification X-ray angiograms of example lungs from 0Gy (control) and 10 Gy irradiated rats at 3-d, 2-wk, 1-m, 2-m, 5-m, and 12-m. Intravascular pressure was set to 12 mmHg and airway pressure 6 mmHg. Scale on bottom-center image indicates 100μm.
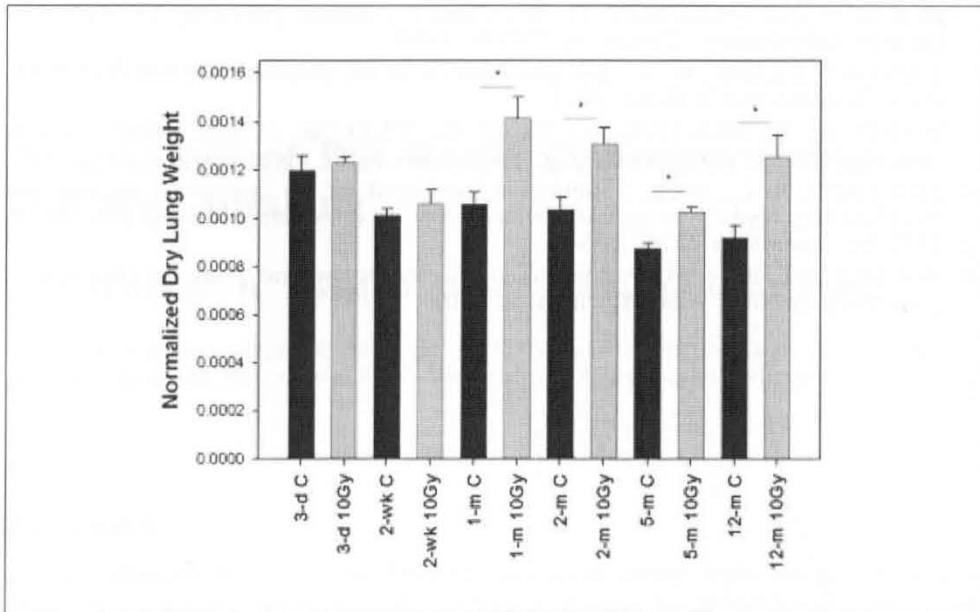
Figure 3. Normalized dry lung weight measured in 0Gy (control) and 10 Gy irradiated rats at 3-d, 2-wk, 1-m, 2-m, 5-m, and 12-m.

increased lung weight and significantly decreased body weight at the 12-m p.i. time point is likely reflective of this later stage process. Supported by Department of Veterans Affairs and NIH U19AI067734.

References

1. STONE, H. B., et al., Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Report of an NCI Workshop, December 3-4, 2003, Radiat Res, 6, 711-28, 2004.

2. AUGUSTINE, A. D., et al., Animal models for radiation injury, protection and therapy, Radiat Res, 1, 100-9, 2005.

3. MOULDER, J. E., Post-irradiation approaches to treatment of radiation injuries in the context of radiological terrorism and radiation accidents: a review, Int J Radiat Biol, 1, 3-10, 2004.

4. MOLTENI, A., et al., Control of radiation-induced pneumopathy and lung fibrosis by angiotensin-converting enzyme inhibitors and an angiotensin II type 1 receptor blocker, Int J Radiat Biol, 4, 523-32, 2000.

5. KWOCK, L., et al., The effects of ionizing radiation on the pulmonary vasculature of intact rats and isolated pulmonary endothelium, Radiat Res, 2, 276-91, 1987.

6. PETERSON, L. M., et al., Vascular response to radiation injury in the rat lung, Radiat Res, 2, 139-48, 1992.

7. RUBIN, P. and CASARETT, G. W., Clinical radiation pathology as applied to curative radiotherapy, Cancer, 4, 767-78, 1968.

8. VAN DEN BRANK, H. A., Radiation effects of the pulmonary systemBaltimore, USA, William and Wilkins, 1971.

9. WARD, W. F., MOLTENI, A., TSAO, C., TAYLOR, J., Endothelial oriented strategies of interventionBoca Raton, FL, USA, CRC Press, 1998.

10. MOLTHEN, R. C., et al., Quantitative models of the rat pulmonary arterial tree morphometry applied to hypoxia-induced arterial remodeling, J Appl Physiol, 6, 2372-84; discussion 2354, 2004.

11. HOLMQUIST, B., et al., A continuous spectrophotometric assay for angiotensin converting enzyme, Anal Biochem, 2, 540-8, 1979.