Age at Exposure to Radiation Determines Severity of Renal and Cardiac Disease in Rats

Authors: Lenarczyk, Marek, Kronenberg, Amy, Mäder, Marylou, North, Paula E., Komorowski, Richard, et al.

Source: Radiation Research, 192(1) : 63-74

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR15043.1

RADIATION RESEARCH **192**, 63–74 (2019)
0033-7587/19 $15.00

DOI: 10.1667/RR15043.1

Age at Exposure to Radiation Determines Severity of Renal and Cardiac Disease in Rats

Marek Lenarczyk,[a] Amy Kronenberg,[e] Marylou Mäder,[b] Paula E. North,[c] Richard Komorowski,[c] Qunli Cheng,[a] Mark P. Little,[f] I-Hung Chiang,[g,1] Chiara LaTessa,[g,2] James Jardine[h] and John E. Baker[a,d,3]

[a] *Division of Congenital Cardiac Surgery and Departments of* [b] *Radiation Oncology,* [c] *Pathology and* [d] *Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin;* [e] *Lawrence Berkeley National Laboratory, Berkeley, California;* [f] *Radiation Epidemiology Branch, National Cancer Institute, Bethesda, Maryland; and* [g] *Collider-Accelerator Department and* [h] *Biology Department, Brookhaven National Laboratory, Upton, New York*

Lenarczyk, M., Kronenberg, A., Mäder, M, North, P. E., Komorowski, R., Cheng, Q., Little, M. P., Chiang, I-H., LaTessa, C., Jardine, J. and Baker, J. E. Age at Exposure to Radiation Determines Severity of Renal and Cardiac Disease in Rats. ***Radiat. Res.*** **192, 63–74 (2019).**

Radiotherapy with sparsely ionizing photons is a cornerstone of successful cancer treatment. Age at time of exposure to radiation is known to influence biological outcomes for many end points. The effect of dose and age at exposure upon the occurrence of radiogenic cardiovascular disease is poorly understood. The goal of this work was to determine the response of male WAG/RijCmcr rats at 6 months of age to gamma rays, and at 6 months or 6 weeks of age to X rays, using clinically relevant biomarkers of cardiovascular disease and kidney injury. Overall, there were significant radiation-induced effects on the levels of bicarbonate ($P = 0.0016$), creatinine ($P = 0.0002$), calcium ($P = 0.0009$), triglycerides ($P = 0.0269$) and blood urea nitrogen, albumin, protein, AST, alkaline phosphatase, total cholesterol and HDL (all $P < 0.0001$). Of those variables with a significant radiation-dose effect, there were significant modifications by age at time of exposure for bicarbonate ($P = 0.0033$), creatinine ($P = 0.0015$), AST ($P = 0.0040$), total cholesterol ($P = 0.0006$) and blood urea nitrogen, calcium, albumin, protein, alkaline phosphatase and HDL (all $P < 0.0001$). Cardiac perivascular collagen content was significantly increased in rats that were 8.0 Gy X-ray irradiated at 6 weeks of age ($P < 0.047$) but not at 6 months of age. While systemic blood pressure was elevated in both cohorts after 8.0 Gy X-ray irradiation (compared to age-matched sham-irradiated controls), the magnitude of the increase above baseline was greater in the younger rats ($P < 0.05$). These findings indicate that dose and age at time of irradiation determine the timeline and severity of cardiac and renal injury.

INTRODUCTION

Radiation is a cornerstone of successful cancer treatment, with one-half to two-thirds of all cancer patients receiving radiotherapy, the vast majority of which involves exposure to sparsely ionizing radiations such as photons (*1, 2*). Total-body irradiation (TBI) is used at a high dose to condition cancer patients prior to transplantation of donor bone marrow stem cells (*3*). Survivors of childhood and adolescent cancer treated with TBI are at increased risk for cardiovascular disease (*4–6*). Total-body irradiation in rats at 5 weeks of age, representative of a pediatric population, increases serum total cholesterol, LDL cholesterol and triglycerides, all of which are clinically accepted biomarkers of increased risk for cardiovascular disease (*7*). Hypercholesterolemia in rats irradiated at 5 weeks of age is associated with morphological injury to cardiac endothelium manifest as perivascular cardiac fibrosis and decreased density of the smaller-diameter coronary vessels (*7*).

Age at time of exposure to sparsely ionizing radiation is known to determine many biological outcomes. Significant modifying effects of age at exposure have been observed for circulatory disease mortality in Japanese atomic bomb survivors (*8*), and some evidence was found for both modifications by age at exposure and attained age in an independent analysis of this data (*9*). In a meta-analysis of radio-epidemiological datasets relating to circulatory disease, Little (*10*) demonstrated significant effects of age at exposure for most circulatory disease end points. Renal tolerance to local kidney irradiation increases with age at time of irradiation (5–25 weeks of age) to maximum tolerance at 15 weeks of age in rats (*11*).

The effect of age at time of exposure to sparsely ionizing radiation upon the occurrence of cardiovascular disease is poorly understood. We reasoned that age at the time of

Editor's note. The online version of this article (DOI: 10.1667/RR15043.1) contains supplementary information that is available to all authorized users.

[1] Deceased.

[2] Current address: University of Trento, Povo, Italy.

[3] Corresponding author: Congenital Cardiac Surgery, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226; email: jbaker@mcw.edu.

exposure to low-LET radiation might determine outcomes for cardiovascular disease in rats. The goal of this study was to determine the response of rats under the following conditions: 1. γ-ray irradiation at 6 months of age; 2. high-energy X-ray irradiation at 6 months of age; and 3. high-energy X-ray irradiation at 6 weeks of age. Radiation response would be determined using clinically significant biomarkers of cardiovascular disease relevant to long follow-up periods in ''rat-equivalent'' time. Because of the link between altered kidney function and cardiovascular risk in young rats, markers of kidney injury were included in this study (*12*).

MATERIALS AND METHODS

Experimental Animals

Male WAG/RijCmcr (Wistar) rats were irradiated at either 6 weeks or 6 months of age. These ages correspond to exposure in late childhood (age of sexual maturity) and middle age in humans. Animals were maintained on Teklad 8904 rat chow (Harlan® Laboratories Inc., Indianapolis, IN) and water *ad libitum* and provided with Sani-Chips® wood chip bedding throughout the study at the Biomedical Resource Center of the Medical College of Wisconsin (MCW, Milwaukee, WI; X-ray studies) or at the Brookhaven National Laboratory (BNL, Upton, NY; γ-ray studies). The rats were maintained on a 12:12 h light-dark schedule at a temperature of 20 ± 1°C and relative humidity of 50–80%. The research was conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The Animal Care and Use Committees at MCW and BNL approved all protocols.

Animal Irradiations

Gamma-ray irradiations. These studies were performed at BNL using a JL Shepherd Mark I γ-ray irradiator (model 68a; San Fernando, CA). Rats were shipped from the MCW to BNL approximately one week in advance to acclimatize prior to γ-ray irradiations. Animals were irradiated or sham-irradiated and shipped back to MCW approximately one week postirradiation and maintained at MCW for the duration of the study. Nonanesthetized 6-month-old rats (n = 12/group) received single-dose TBI (1.5, 3.0 or 6.0 Gy ^{137}Cs; 662 keV, LET approximately 0.8 keV/micron). Total-body irradiation was performed using a lateral field with a dose rate of 54.02 cGy/min at the midline of the animal. These γ-ray doses do not require bone marrow transplantation to maintain the general health of the animals. Rats were immobilized in Plexiglas® restraint jigs with one flank facing the line source. Each irradiation jig was rotated horizontally 180° halfway through the irradiation procedure to ensure approximate dose uniformity. Sham-irradiated rats, serving as controls, were also immobilized in Plexiglas restraint jigs (n = 12/group) for the same amount of time required for an average irradiation. Room air was drawn through the interior of the irradiator using an external vacuum line to maintain oxygen levels at 21% within the chamber that houses the rats, and to remove ozone. A battery-powered light was placed within the irradiator to maintain illumination inside the chamber during the time the door was closed.

Supplemental gamma-ray dosimetry. These studies were performed at BNL using the JL Shepherd Mark I γ-ray irradiator, model 68a ^{137}Cs source. Three positions were available for placement of samples at different radial distances from the line source and within the lead shielding cabinet provided by the manufacturer. For these studies, additional dosimetry was performed at position no. 3 to simulate the planned rat irradiations. Measurements were taken with the dosimetry set up (jig with detector) at a height of 17.78 cm above the floor of the cabinet and with the use of the standard positioning platform to ensure stability and reproducibility. Measurements were taken with the 2× attenuator in place to deliver a dose rate of 54.02 cGy/min.

The dose was measured with an EG&G thimble ionization chamber (Goleta, CA) placed at different positions inside a Plexiglas restraint jig designed for rat irradiations. The jig had a wall thickness of 0.635 cm. The ionization chamber was cross-calibrated to a National Institute of Standards and Technology-traceable source to provide absolute dose values, and its reproducibility was <1% if the detector was maintained in place for replicate measurements. Dosimetry was performed with the central point of the long axis of the jig at position no. 3 within the lead shielding, reproducing the geometry that was planned for the rat irradiations. A central point was chosen for a ''standard'' rat placed within the Plexiglas jig to define the point of ''100%'' of measured dose. A relative exposure of 93.6% was measured at the ''nose'' position of the rat inside the restraint jig, with a relative exposure of 94.4% at the ''tail'' position within the restraint jig, considering the Plexiglas backplate used for positioning purposes.

X-ray irradiations. These studies were performed using an X-RAD X-ray irradiator (Precision X-Ray Inc., Branford, CT) at MCW. Nonanesthetized 6-week-old and 6-month-old rats received single-dose TBI (6.0, 8.0 or 10.0 Gy; n = 8/group). Total-body irradiation was performed using a posterior-anterior field at a dose rate of 50 cGy/min, with 320 kVp orthovoltage X rays. The half-value layer of the beam used for irradiation was 1.4 mm of copper for rats receiving 8.0 and 10.0 Gy TBI, the left hind leg was placed outside the radiation field to avoid the need for bone marrow transplantation. The radiation dosimetry has been described in detail elsewhere (*13*). Rats were immobilized in Plexiglas restraint jigs throughout the irradiation period. Rats of the same age (6 weeks or 6 months) were sham-irradiated in Plexiglas jigs (n = 8/group) to serve as controls. Rats were maintained after irradiation in microisolator cages for the duration of the study.

Cardiac Risk Factors

Blood was drawn by venipuncture from the jugular vein at 30-day intervals beginning 30 days postirradiation and continuing up to 270 days post-TBI. Blood was also taken from sham-irradiated control rats at the same time intervals. Serum was then analyzed for total cholesterol, HDL cholesterol and triglycerides (Wisconsin Diagnostic Laboratories, Milwaukee, WI).

Kidney Injury and Metabolic Biomarkers

Blood was withdrawn by venipuncture from the jugular vein at 30-day intervals beginning at day 30 postirradiation and continuing up to 270 days post-TBI, and from sham-irradiated control rats. Serum was analyzed for blood urea nitrogen (BUN), creatinine, total protein and albumin, and for electrolyte and fluid balance, liver function and glucose (Wisconsin Diagnostic Laboratories, Milwaukee, WI).

Systemic blood pressure (systolic and diastolic) was measured using a non-invasive photoelectric tail-cuff system (Visitech Systems, Apex, NC) at 90-day intervals starting at day 90 postirradiation and up to 270 days postirradiation. Mean blood pressure was calculated from systolic and diastolic values. Nonanesthetized rats were placed in plastic restrainers. A cuff with a pneumatic pulse sensor was attached to the tail. Rats were allowed to habituate to this procedure for 7 days before blood pressure measurements were performed. Blood pressure values were recorded without heating and were averaged from at least three consecutive readings obtained from each rat. Blood pressure and heart rate of rats were measured between 1:00 pm–5:00 pm for all experiments.

Histology

To evaluate tissue damage at 270 days postirradiation, the entire heart and two kidneys (n = 6/group) were removed from fully

TABLE 1
Experimental Design

Radiation source	Age at irradiation	Dose	Group size
^{137}Cs γ rays	6 months	1.5, 3.0, 6.0 Gy	12
X rays	6 weeks	6.0, 8.0, 10.0 Gy	8
X rays	6 months	6.0, 8.0, 10.0 Gy	8

anesthetized TBI and sham-irradiated (control) rats and fixed in 10% formalin (v/v) using our standard procedures, as described elsewhere (*14, 15*). Prior to embedding, the heart was oriented between the base and the apex, and the kidneys oriented along the mid-dorsal plane. Fixed tissue samples were embedded in paraffin with kidney samples in coronal orientation and heart samples embedded in transverse plane. Sections, 4 μm thick, were cut from each block and stained with hematoxylin and eosin (H&E) or Masson-trichrome according to standard methods described elsewhere (*14, 15*). Ten sections from each heart and kidney were used for morphometric analysis as described elsewhere (*12*).

Experimental Design and Statistical Analysis

The numbers in each experimental group were derived from a power analysis based on our previous experience with the same measurements in similar studies. Animals were randomized to each group. The identity of the animal in each experimental group was known to the investigator responsible for initiating and continuing with an intervention, for example, irradiation. The identity of the animal under study was not known to the investigator performing the experimental measurement or the analysis. These investigators were not the same person. Cardiac fibrosis in the perivascular region and vessel wall was expressed as a percentage of vessel luminal area. The study tested the hypothesis that age at time of radiation exposure determines cardiac outcomes. The start of the study was defined as the time rats were irradiated or sham-irradiated. All values were expressed as the mean ± standard deviation. All statistical analyses were performed using SigmaPlot® version 11.0 software. Exploratory data analysis was performed using a Shapiro-Wilk test (*16*) followed by unpaired Student's *t* test for two-group comparisons. Data failing the Shapiro-Wilk test of normality (*16*) were analyzed using the Mann-Whitney rank sum (U) test (*17*).

For regression analysis of various blood-measured cardiac end points, a linear random effects model was used, with random effect of animal, to adjust for the within-individual correlations. Models were fitted using the lmer function in the lme4 package (*18*) using R (*19*). Sensitivity analyses were also performed using linear models without random effects, reported in Appendix Table A1. The customary threshold for statistical significance ($P < 0.05$) was used in the analysis. The experimental design is shown in Table 1. Individual *P* values at specific time points in the longitudinal studies are reported in the figures.

RESULTS

Radiation Dose Response for Blood-Measured End Points across all Cohorts

The distribution of various blood-measured parameters that may influence the risk of cardiac disease after photon irradiation is shown in Table 2. The results were analyzed using the random effects model and are shown in Table 3. Overall, dose-dependent changes were evident in several key indicators of cardiac risk when the three photon-irradiated cohorts were pooled.

Cardiac end points. There were highly significant ($P < 0.0001$) age-adjusted increases of total cholesterol and HDL cholesterol with increasing photon dose, and significant increases ($P = 0.0269$) also of triglycerides (see Table 3, column 2). However, the total cholesterol/HDL ratio did not significantly vary with dose ($P = 0.1643$) (Table 3, column 2). Models without a random effects term per animal yielded very similar results (Appendix Table A1).

Kidney end points. An examination of kidney-relevant end points revealed highly significant age-adjusted increases in BUN ($P < 0.0001$) and creatinine ($P = 0.0002$) with increasing photon dose (Table 3, column 2). In contrast, there were significant age-adjusted decreases ($P < 0.0001$) with radiation dose for total protein and albumin. Models without random effects term per animal yielded very similar results (Appendix Table A1).

Other end points measured in blood. The analysis shown in Table 3 column 2 further demonstrates that after adjustment for age there were highly significant increases

TABLE 2
Description of Distribution of Experimentally Measured Parameters

	Mean (minimum, maximum)	1st quartile/median/3rd quartile
Sodium (mmol/l)	143.58 (127.00, 150.00)	143.00/144.00/145.00
Potassium (mmol/l)	5.67 (3.50, 27.30)	5.00/5.30/5.50
Chlorine (mmol/l)	97.06 (86.00, 102.00)	96.00/97.00/98.00
Bicarbonate (mmol/l)	25.25 (17.00, 36.00)	24.00/25.00/27.00
Glucose (mg/dl)	95.79 (2.00, 253.00)	86.00/97.00/106.00
BUN (mg/dl)	21.68 (14.00, 140.00)	18.00/19.00/20.00
Creatinine (mg/dl)	0.38 (0.20, 2.29)	0.33/0.36/0.39
Calcium (mg/dl)	10.47 (9.10, 12.10)	10.20/10.50/10.70
Albumin (g/dl)	4.37 (2.80, 6.30)	4.20/4.40/4.60
Total protein (g/dl)	6.37 (3.60, 7.50)	6.20/6.50/6.70
AST (IU/I)	97.68 (3.00, 237.00)	80.00/92.00/111.00
Alkaline phosphatase (IU/I)	133.52 (52.00, 321.00)	116.00/128.00/140.00
ALT (IU/I)	52.36 (6.30, 169.00)	47.00/51.00/57.00
Total cholesterol (mg/dl)	97.42 (49.00, 225.00)	79.00/92.00/107.00
HDL cholesterol (mg/dl)	86.53 (45.00, 207.00)	70.00/82.00/94.25
Total cholesterol/HDL ratio	1.13 (0.94, 1.46)	1.07/1.12/1.17
Triglycerides (mg/dl)	132.84 (40.00, 382.00)	100.00/125.00/159.00

TABLE 3
Linear Mixed Effect (Random Effect) Model Fitted to Various End Points

	P value for age effect[a]	*P* value for dose effect[b]	*P* value of age at exposure x dose[c]	Dose effect (/Gy) (+95% CI)[d]	Dose × age at exposure effect (/Gy/100 days) (central + 95% CI)[d]
Sodium (mmol/l)	<0.0001	0.5413	0.1051	–0.029 (–0.087, 0.030)	–0.075 (–0.165, 0.016)
Potassium (mmol/l)	0.0278	0.4514	0.8948	–0.033 (–0.092, 0.026)	–0.006 (–0.097, 0.085)
Chlorine (mmol/l)	0.0108	0.7275	0.1907	–0.006 (–0.053, 0.041)	–0.048 (–0.120, 0.024)
Bicarbonate (mmol/l)	<0.0001	0.0016	0.7379	0.082 (0.024, 0.140)	–0.016 (–0.103, 0.072)
Glucose (mg/dl)	0.4520	0.1349	0.0056	0.048 (–0.527, 0.624)	–1.252 (–2.135, –0.365)
BUN (mg/dl)	0.0167	<0.0001	0.0068	0.921 (0.483, 1.359)	–0.912 (–1.566, –0.257)
Creatinine (mg/dl)	<0.0001	0.0002	0.1289	0.008 (0.002, 0.013)	–0.007 (–0.015, 0.002)
Calcium (mg/dl)	<0.0001	0.0009	0.0299	–0.022 (–0.033, –0.011)	–0.019 (–0.035, –0.002)
Albumin (g/dl)	<0.0001	<0.0001	0.0003	–0.039 (–0.052, –0.026)	0.037 (0.018, 0.057)
Total protein (g/dl)	0.4899	<0.0001	0.0014	–0.059 (–0.074, –0.044)	0.038 (0.015, 0.061)
AST (IU/I)	<0.0001	<0.0001	0.0014	–0.749 (–1.329, –0.169)	1.462 (0.568, 2.357)
Alkaline phosphatase (IU/I)	<0.0001	<0.0001	0.0003	–1.795 (–2.728, –0.861)	2.633 (1.220, 4.045)
ALT (IU/I)	<0.0001	0.0565	0.0598	–0.029 (–0.554, 0.497)	0.592 (–0.025, 1.210)
Total cholesterol (mg/dl)	<0.0001	<0.0001	0.0008	2.791 (1.763, 3.818)	–2.652 (–4.183, –1.119)
HDL cholesterol (mg/dl)	<0.0001	<0.0001	0.0030	2.701 (1.828, 3.574)	–1.995 (–3.298, –0.691)
Total cholesterol/HDL ratio	<0.0001	0.1643	0.0883	–0.002 (–0.004, 0.000)	–0.003 (–0.006, 0.000)
Triglycerides (mg/dl)	<0.0001	0.0269	0.0333	1.140 (–0.734, 3.014)	–3.057 (–5.866, –0.248)

[a] *P* value of improvement in fit of a frailty model with random effect (per rat) and age at blood draw (days) vs. model with random effect (per rat) only.

[b] *P* value of improvement in fit of a frailty model with random effect (per rat), age at blood draw (days) and radiation dose (Gy) vs. model with random effect (per rat) and age at blood draw (days) only.

[c] *P* value of improvement in fit of a frailty model with random effect (per rat), age at blood draw (days), radiation dose (days), centered age at exposure (days) and radiation dose x (centered age at exposure) vs. model with random effect (per rat), age at blood draw (days), centered age at exposure (days) and dose (Gy) only.

[d] Taken from frailty model with random effect (per rat), age at blood draw (days), radiation dose (Gy), centered age at exposure (days) and radiation dose x (centered age at exposure).

in blood levels of bicarbonate ($P = 0.0016$) with increasing photon dose when data from all cohorts were considered together. In contrast, increased radiation dose was associated with highly significant decreases in blood calcium ($P = 0.0009$), AST ($P < 0.0001$) and alkaline phosphatase ($P < 0.0001$) for the combined cohorts. There were no significant age-adjusted changes for sodium ($P = 0.5413$), potassium ($P = 0.4514$), chlorine ($P = 0.7275$), glucose ($P = 0.1349$) or ALT ($P = 0.0565$) with radiation dose (Table 3, column 2). Models without a random effects term per animal yielded very similar results (Appendix Table A1).

Interaction of Dose and Age at Exposure for Various Blood-Measured Cardiac End Points across all Cohorts

Age at exposure is one of the principal factors to consider when examining radiation dose-response relationships. The data acquired for the three rat cohorts irradiated with high-energy photons (one at 6 weeks of age, two at 6 months of age) revealed modifying effects of age at exposure for several important end points. Of those end points with a significant radiation dose response (whether positive or negative), there were significant augmentations of the dose response with increasing age at time of irradiation (i.e., increasing age at time of irradiation led to a more strongly positive dose response) for albumin ($P = 0.0003$), protein ($P = 0.0014$), AST ($P = 0.0014$) and alkaline phosphatase ($P = 0.0003$) (see Table 3, column 3). This is in contradistinction to significant diminutions of the dose response with increasing age at time of irradiation (i.e., increasing age at time of irradiation led to a more strongly negative dose response) for major parameters associated with cardiac risk including total cholesterol ($P = 0.0008$), HDL ($P = 0.0030$), triglycerides ($P = 0.0333$), BUN ($P = 0.0068$) and calcium ($P = 0.0299$) (Table 3, column 3). Models without a random effects term per animal yielded very similar results (Appendix Table A1).

Radiation Response in Gamma-Ray Irradiated 6-Month-Old Rat Cohort

There were no deaths among the sham-irradiated rats. One of 12 rats died at 174 days after 1.5 Gy irradiation and one of 12 rats died at 238 days after 6.0 Gy irradiation.

Cardiac disease indicators. Total cholesterol, HDL cholesterol and triglyceride levels in sham-irradiated rats progressively increased over the 270-day study period compared to values at 30 days (Supplementary Fig. S1; http://dx.doi.org/10.1667/RR15043.1.S1) but each was essentially unchanged in γ-ray-irradiated rats when compared to age-matched sham-irradiated controls. Even at the highest dose of 6.0 Gy γ-ray irradiation, the coronary vessels and cardiomyocytes from rats remained normal in appearance compared to the hearts from age-matched, sham-irradiated rats (Supplementary Fig. S2). There was no increase in cardiac perivascular collagen deposition in

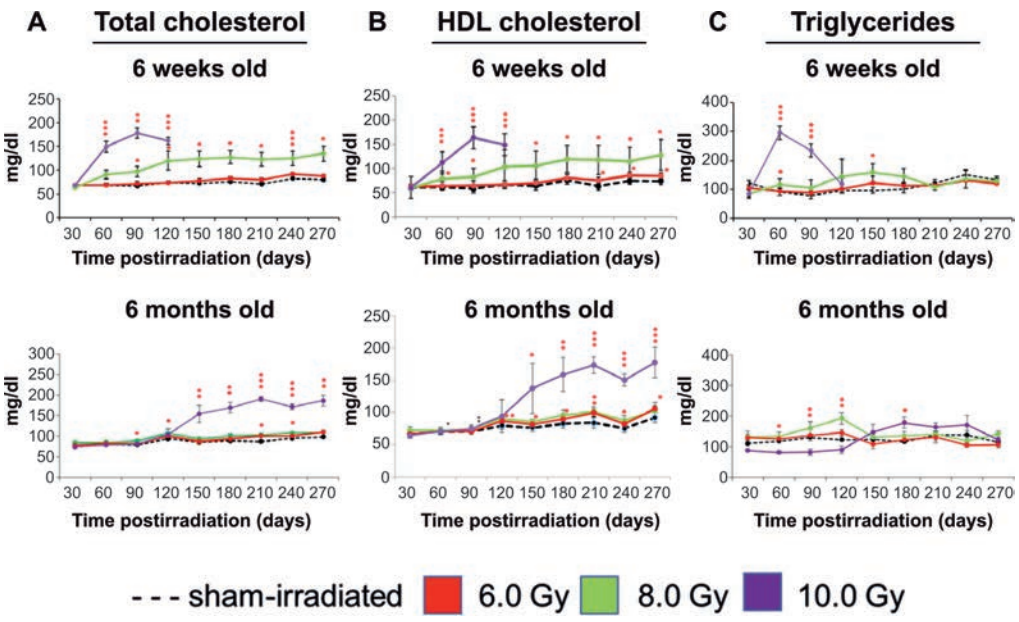

FIG. 1. Risk factors for cardiac disease after X-ray irradiation of rats at 6 weeks (upper panels A–C) and 6 months of age (lower panels A–C). Total cholesterol, HDL cholesterol and triglycerides after 6.0, 8.0 or 10.0 Gy X-ray irradiation of rats at 6 weeks and 6 months of age. Data are mean ± SD, n = 8/group. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ when compared to age-matched sham-irradiated control.

irradiated hearts compared to age-matched sham-irradiated controls.

Kidney injury indicators. BUN and creatinine levels were largely unchanged in sham-irradiated rats over the 270-day study period, and these levels were also unchanged over the 270-day follow-up period for all γ-ray-irradiated rats (Supplementary Fig. S3; http://dx.doi.org/10.1667/RR15043.1.S1). Similarly, total protein and albumin were essentially unchanged in γ-ray-irradiated rats compared to controls (Supplementary Fig. S3). Cortex and medulla from rats at 270 days after 6.0 Gy γ-ray irradiation remained normal in appearance compared to kidneys from age-matched, sham-irradiated rats (Supplementary Fig. S4). Systemic blood pressure (systolic and diastolic) was not significantly elevated over the 270-day period after total-body γ-ray irradiation (Supplementary Fig. S5).

Radiation Response in X-Ray Irradiated (320 kVp) 6-Month-Old Rat Cohort

There were no deaths associated with sham-irradiated rats, nor were there any deaths associated with X-ray irradiation at any dose.

Cardiac disease indicators. Total- and HDL cholesterol levels in sham-irradiated rats gradually and significantly increased over the 270-day study period (Fig. 1A–C, lower panels). Irradiation with 6.0 Gy of 320 kVp X rays did not significantly increase total cholesterol, HDL cholesterol or triglyceride levels over the 270-day study period, supporting the results obtained from the 6-month-old rats that received 6.0 Gy γ-ray irradiation (above). X-ray irradiation with 8.0 Gy slightly but significantly ($P < 0.05$) increased total cholesterol late in the study period (210–240 days), but this level returned to those of the age-matched controls at 270 days. HDL cholesterol was increased slightly but significantly at 120–270 days. In contrast to the later changes in cholesterol levels, triglycerides were significantly elevated at early time points, 60120 days after 8.0 Gy irradiation, then declined to control values. Notably, 10.0 Gy irradiation significantly increased total- and HDL cholesterol levels starting after 120 days, and both total- and HDL cholesterol levels remained significantly elevated over the next 120 days. Triglyceride levels were also significantly elevated in the early response to 10.0 Gy, after 60 and 90 days, and at later times the triglyceride levels declined to control levels, following a somewhat similar pattern to the response at 8.0 Gy irradiation.

Histological sections were analyzed after 270 days by two cardiac pathologists blinded to the identity of the specimens (PEN and RK). Histological analysis revealed that hearts from 6-month-old sham-irradiated rats had similar symmetrical penetrating coronary vessels and showed no accumulation of amphophilic material (Fig. 2). In 6-month-old 8.0 Gy irradiated rats, there was no significant increase in cardiac perivascular collagen content compared to age-matched, sham-irradiated controls (Fig. 2).

Hearts from 6-month-old 10.0 Gy irradiated rats were not examined, because our goal was to determine age-related changes in radiation response after 270 days. Since young rats irradiated with 10.0 Gy at 6 weeks of age needed to be euthanized after 120 days, in compliance with IACUC requirements, we did not have a suitable control set of 6-month-old animals to examine at the 120-day time point (see below).

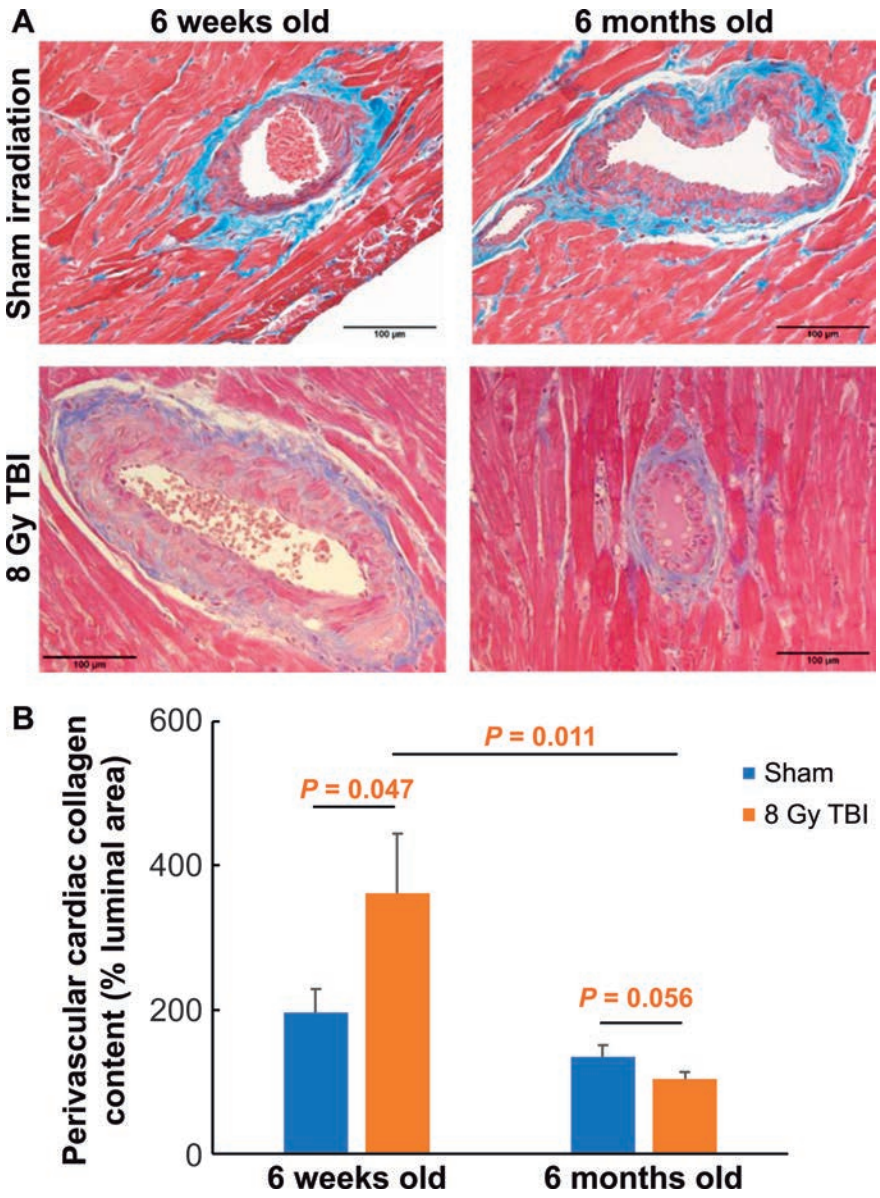

FIG. 2. Morphological changes to heart after X-ray irradiation of rats at 6 months and 6 weeks of age. Morphological changes to heart 270 days after 8.0 Gy X-ray irradiation. Panel A: Heart sections stained with trichrome. Panel B: Measurement of perivascular cardiac collagen content, an index of cardiac fibrosis. Data are mean ± SD, n = 6/group.

Kidney injury indicators. In rats that were 6 months old at the start of the study, BUN, creatinine, total protein and albumin levels were unchanged for the sham-irradiated group over the 270-day study period (Fig. 3A–D, lower panels). X-ray irradiation of 6-month-old rats with 6.0 or 8.0 Gy did not alter BUN or creatinine levels over the 270-day study period (Fig. 3A–D, lower panels). Total protein and albumin levels were essentially unchanged in rats after 6.0 Gy irradiation. X-ray irradiation (8.0 Gy) of 6-month-old rats intermittently but significantly ($P < 0.05$) decreased blood total protein and albumin. In contrast, 10.0 Gy irradiation consistently and significantly decreased total protein and albumin levels over the 270-day study period and increased BUN starting at 150 days postirradiation to 240 days. The BUN measurement in the 10.0 Gy irradiated group was significantly elevated at 270 days [$P = 0.008$ (or $P < 0.01$)].

An examination of the kidneys from animals irradiated at 6 months of age did not demonstrate major changes in morphology after either 6.0 or 8.0 Gy irradiation. The glomerular endothelium was intact regardless of age at time of irradiation or radiation dose. In rats that were 6.0 or 8.0 Gy irradiated at 6 months of age, there was no morphological injury to the nephron nor was there any glomerular sclerosis in the tubules (Fig. 4).

Systemic blood pressures were stable in the 6-month-old sham-irradiated cohort over the 270-day study period (Fig. 5). In 6-month-old rats irradiated with 6.0 or 8.0 Gy,

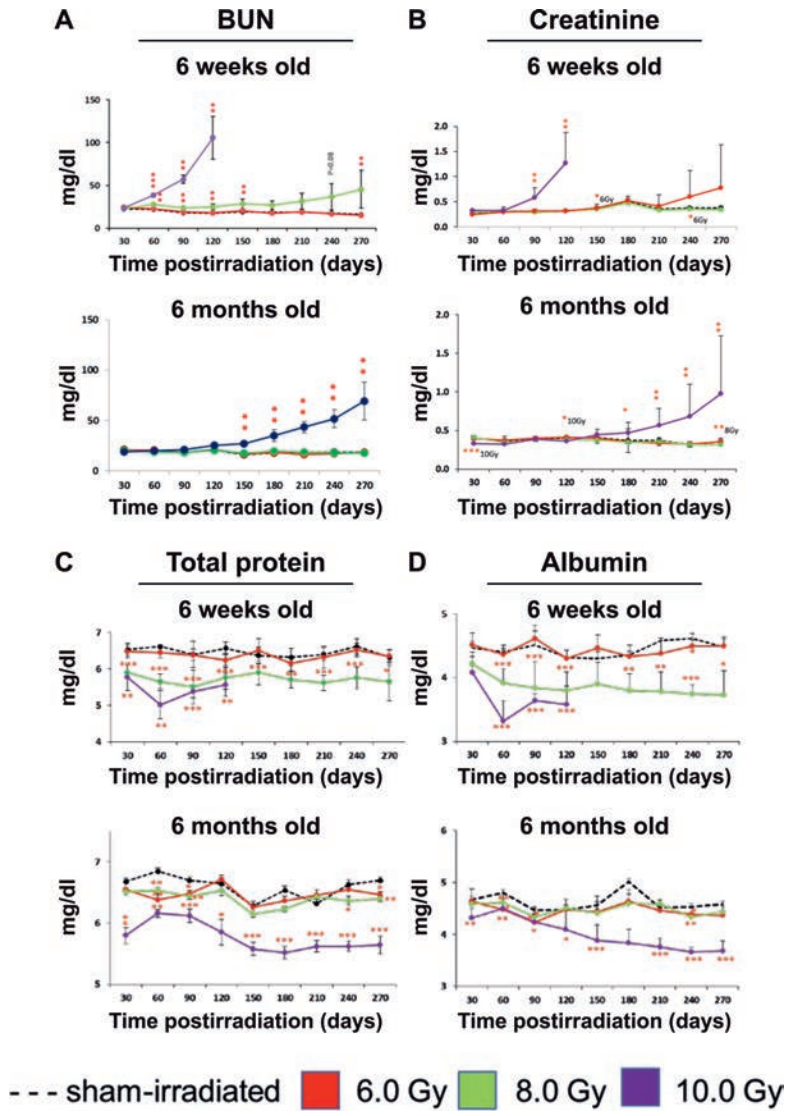

FIG. 3. Kidney injury after X-ray irradiation of rats at 6 weeks (upper panels A–D) and 6 months of age (lower panels A–D). BUN, creatinine, total protein and albumin after 6.0, 8.0 or 10.0 Gy X-ray irradiation. Data are mean ± SD, n = 8/group. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ when compared to age-matched control.

systolic, mean and diastolic blood pressures were significantly elevated at 270 days after the start of the study. In contrast, 10.0 Gy irradiation elevated systolic, mean and diastolic blood pressures at 180 and 270 days after the start of the study. The extent of hypertension was greater after 10.0 Gy irradiation compared to 6.0 and 8.0 Gy (Fig. 5).

Radiation Response in X-Ray Irradiated (320 kVp) 6-Week-Old Rat Cohort

There were no deaths associated with sham-irradiated rats. One of 8 rats died at 180 days after 8.0 Gy irradiation. We note that the study period was limited to 120 days for 6-week-old 10.0 Gy irradiated rats, for reasons described below.

Cardiac disease indicators. In rats 6 weeks of age at the start of the study, total cholesterol, HDL cholesterol and triglyceride levels in the sham-irradiated group gradually and significantly increased over the 270-day study period (Fig. 1A–C, upper panels). Irradiation of young rats with 6.0 Gy of 320 kVp X rays did not significantly increase total cholesterol or triglyceride levels over the 270 -day follow-up period. HDL cholesterol levels in rats that were 6.0 Gy X-ray irradiated were slightly but significantly ($P < 0.05$) increased after 210 days compared to age-matched sham-irradiated controls (Fig. 1A–C, upper panels). X-ray irradiation of young rats with 8.0 or 10.0 Gy increased total cholesterol levels starting as early as 60 days postirradiation, and these levels remained significantly elevated over the duration of the study, in sharp contrast to the results for rats irradiated at 6 months of age. Overall, the timing of the appearance of changes in total cholesterol was earlier in the younger animals, and the magnitude of elevation in total

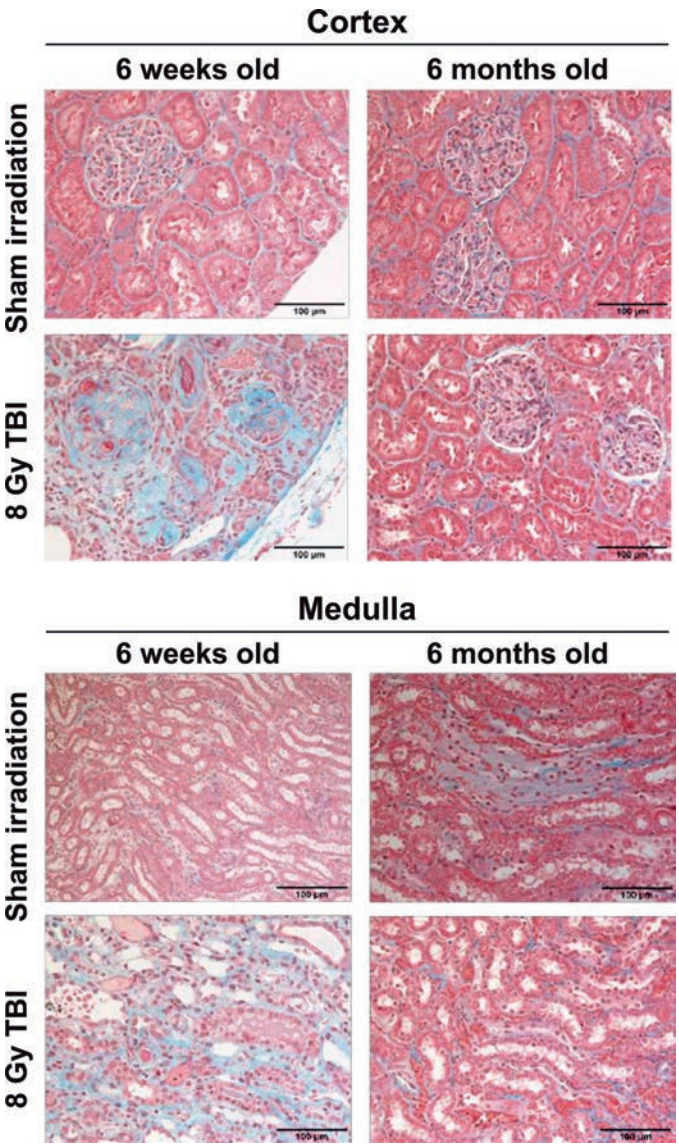

FIG. 4. Morphological changes to kidney after X-ray irradiation of rats at 6 weeks and 6 months of age. Kidney cortex and medulla stained with trichrome 270 days after 8.0 Gy X-ray irradiation.

cholesterol was substantially and significantly greater in rats irradiated at 6 weeks of age compared to rats irradiated at 6 months of age. Elevations in total cholesterol were minimized and/or greatly delayed after irradiation of 6-month-old rats compared to 6-week-old rats. Furthermore, triglyceride levels were markedly and significantly elevated at the 60- and 90-day sampling times after 10.0 Gy irradiation but returned to the level of sham-irradiated animals at 120 days (Fig. 1A–C, upper panels).

Hearts from 6-week-old sham-irradiated control rats had penetrating arteries showing no medial fibrosis or accumulation of amphophilic material (Fig. 2). In 6-week-old rats, 8.0 Gy TBI resulted in an increase in peri-arterial sclerosis of small caliber penetrating coronary vessels with irregular deposition of collagen at the final harvest time of 270 days, compared to age-matched sham-irradiated controls (Fig. 2). Affected vessels had partial to complete luminal sclerosis due to concentric laminar thickening of the vessel walls from the accumulation of amphophilic matrix material between layers of hyperplastic and vacuolated smooth myocytes. Cardiomyocytes from rats that received TBI remained normal in appearance (Fig. 2). Quantification of trichrome staining for perivascular collagen deposition showed significant ($P = 0.047$) cardiac fibrosis in rats irradiated at 6 weeks of age. In contrast, there was no significant ($P = 0.056$) cardiac fibrosis in the vessel wall of hearts from rats irradiated at 6 months of age (Fig. 2). Young rats irradiated with 10.0 Gy were euthanized after 120 days in compliance with IACUC requirements (see below). Trichrome staining of hearts retrieved at this time point showed significantly increased perivascular collagen deposition around the smaller diameter coronary vessels compared with age-matched sham-irradiated controls, confirming our previously published findings (*7*) after 10.0 Gy TBI (results not shown).

Kidney injury indicators. Assessment of kidney parameters in 6-week-old rats indicated that BUN, creatinine, total protein and albumin levels did not vary significantly in the sham-irradiated group over the 270-day study period (Fig. 3A–D, upper panels). Similarly, total-body 6.0 Gy X-ray irradiation of 6-week-old rats did not significantly alter BUN or creatinine levels (Fig. 3A–D, upper panels). In contrast, X-ray irradiation of 6-week-old rats with 8.0 or 10.0 Gy significantly increased BUN levels, and this increase was evident early in the follow-up period, starting at day 60 postirradiation, with the extent of the increase greater after both 8.0 and 10.0 Gy irradiation. In 10.0 Gy irradiated rats, BUN levels exceeded 120 mg/dl after 120 days. These animals were euthanized in accordance with animal care and use regulations at MCW. Irradiation with 8.0 or 10.0 Gy decreased total protein and albumin, with the extent greater in the 10.0 Gy irradiated animals (Fig. 3). However, creatinine levels were increased in only the 6-week-old rats after 10.0 Gy irradiation.

Histological analysis of kidneys revealed that age was not a major factor in the gross appearance of sham-irradiated animals. In the sham-irradiated rats from both the 6-month-old and 6-week-old irradiated cohorts, kidney cortex and medulla were normal and mature in appearance with no macroscopic and microscopic alterations at the end of each 270-day study period (Fig. 4). There was no observed fibrosis.

In rats that were 6.0 Gy irradiated at 6 weeks of age, there was no morphological injury to the nephron after 270 days. In contrast, in young rats that were 8.0 Gy irradiated, there was injury to the cortex, detected as fibrosis, degeneration and mesangiolysis of the glomeruli, and injury to the medulla, detected as fibrosis in the tubules after 270 days. The glomerular endothelium was disrupted in irradiated rats (Fig. 4). It was necessary to euthanize the young 10.0 Gy irradiated rats after 120 days of follow-up. Trichrome

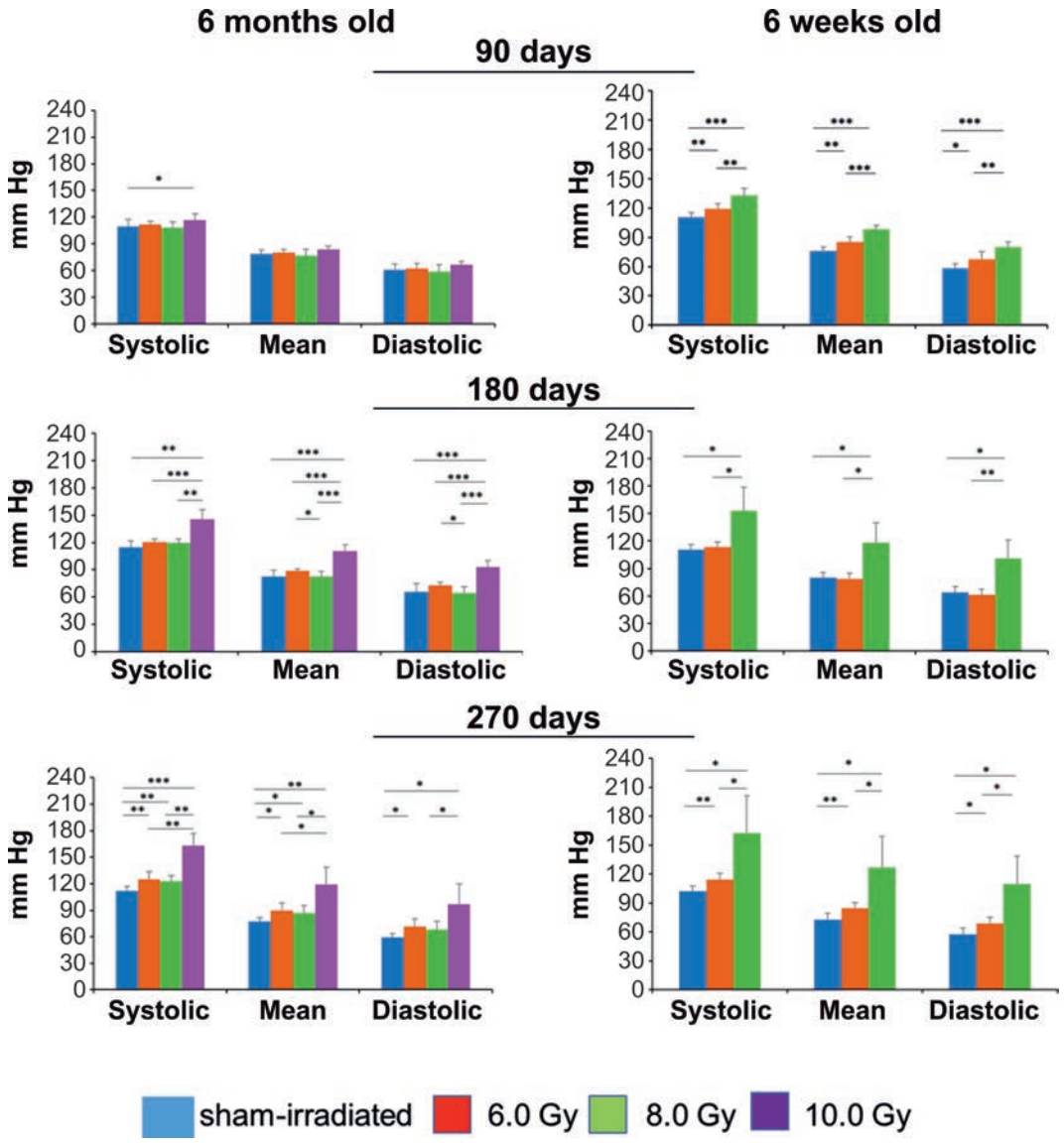

FIG. 5. Systemic blood pressure after X-ray irradiation of rats at 6 months and 6 weeks of age. Systolic, mean and diastolic pressure of rats after 6.0, 8.0 or 10.0 Gy X-ray irradiation. Data are mean ± SD, n = 8/group. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

staining of the kidneys showed glomerulosclerosis (results not shown).

Systemic blood pressures were stable in 6-week-old sham-irradiated groups over the 270-day study period (Fig. 5). In contrast to the results for the 6-month-old irradiated rats, 6-week-old rats irradiated with 6.0 or 8.0 Gy demonstrated elevated systolic, mean and diastolic blood pressures much earlier, starting at 90 days after the start of the study, although the increase for the 6.0 Gy irradiated animals was modest and variable with time. Blood pressures remained significantly elevated in the younger cohort at 180 days and 270 days after 8.0 Gy irradiation (Fig. 5). The extent of hypertension was age- and dose-dependent, increasing with the dose of radiation. Blood pressure in the 10.0 Gy irradiated cohort after 90 days was unable to be measured due to technical problems with the recording system. Our previously reported studies showed systemic blood pressure was increased 120 days after 10.0 Gy TBI (*7*).

DISCUSSION

The overall results obtained in the current study of male rat cohorts that received TBI with conventional photons (Cs γ rays or high-energy X rays) demonstrate modifications in well-accepted risk factors for cardiac disease (e.g., total cholesterol, HDL cholesterol, triglycerides) and kidney injury (e.g., BUN, creatinine, albumin, protein) over a dose range up to 10.0 Gy. In addition to the conventional risk factors that showed significant dose responses (e.g., blood urea nitrogen, albumin and total cholesterol), some novel and potentially interesting dose-dependent results were

obtained for other parameters measured (e.g., bicarbonate and calcium). However, the key finding of this study is that there is a shift in the timing and the magnitude of increased risk factors for cardiac disease and kidney injury in male rats that is dependent on the age at time of irradiation. For 6-month-old male Wistar rats, 1.5–6.0 Gy γ-ray irradiation or 6.0 Gy X-ray irradiation did not increase risk factors for cardiac disease (total cholesterol, HDL cholesterol and triglycerides), the occurrence of cardiac disease (peri-arterial cardiac fibrosis) or kidney injury (BUN, creatinine, total protein, albumin, systemic blood pressure and morphology) over the 270-day follow-up period. Notably, 6-month-old rats that were X-ray irradiated with 8.0 or 10.0 Gy did demonstrate dose-dependent changes in cardiac risk factors and kidney function; however, these changes did not appear early in the 270-day follow-up period. In contrast, X-ray irradiation of male Wistar rats at 6 weeks of age with 8.0–10.0 Gy revealed increased risk factors for cardiac disease and kidney injury in a dose-dependent manner, and these changes appeared within the first two months postirradiation. There was also a modest indication of change in some cardiac risk factors for young animals that received 6.0 Gy irradiation (e.g., blood pressure). The increase in risk factors for cardiac disease and kidney injury in rats that received 10.0 Gy TBI at 6 weeks of age confirms our previously published findings, while the current study extends these observations to lower TBI doses (*7*). In summary, changes in serum cholesterol, HDL cholesterol, BUN and creatinine are manifest earlier and in a more robust manner in male rats receiving 8–10.0 Gy irradiation at 6 weeks old compared to those at 6 months old.

Our finding, that age at exposure to radiation determines severity of heart disease in rats, is comparable to outcomes observed in Japanese atomic bomb survivors. In a model of risk of death from circulatory disease adjusted for age at exposure in atomic-bomb survivors, there is a trend towards reduction in risk of radiation-induced death from 20.73%/Sv at $\leq$9 years of age at exposure to 2.05%/Sv at $\geq$70 years of age at exposure (*9*). Comparisons between the two studies are made with caution as the atomic bomb explosions generated a mixed beam of γ rays and neutrons (*20, 21*) compared to the γ rays alone or X rays alone used in the current study. However, the proportion of the total radiation dose from γ rays, accounting for the greater biological effectiveness of neutrons relative to γ rays (*22, 23*) is 80–90% in the Japanese atomic bomb explosions. Similarly, in a study of 326 patients who received mantle irradiation, the observed-to-expected ratio of increased risk for fatal myocardial infarction was 38.2 in patients treated before the age of 21 compared to only 2.8 in all patients (*24*). These human TBI data on increased cardiac risk to younger individuals exposed to photons add relevance to our findings of increased susceptibility of younger rats exposed to photons.

The end points used in the current study are directly relatable to clinically relevant markers of human cardiovascular diseases and to significant, late disease pathology. Biomarkers used in our study follow the current standard-of-care clinical practices, and include Framingham risk factors such as blood cholesterol, kidney function markers, blood pressure, and histological markers of cardiac and renal fibrosis. In the current study, we measured a total of 12 phenotypes to determine risk for and occurrence of cardiac disease and kidney injury. The profiles for serum levels of HDL cholesterol in this rat study track closely with the profile for total cholesterol. Species differences exist in the distribution of HDL cholesterol between rat and human. Serum total cholesterol in rats is comprised of ~90% HDL cholesterol, while serum total cholesterol in humans is comprised of ~40% HDL cholesterol. This difference is due in part to rat serum containing very little LDL cholesterol compared to human serum (*25*).

Our studies suggest that cardiac injury after TBI is associated with injury to the kidney. The kidney is known to be a radiosensitive organ, susceptible to the development of nephropathy, proteinuria and hypertension after irradiation (*26*). Renal dysfunction has been proposed as part of the mechanism causing increased cardiac disease in cancer survivors treated with radiation (*27*) and in survivors of atomic bombs (*28*). Glomerular filtration rate reaches adult levels by approximately 7 weeks of age in Wistar rats, indicating that the kidney is mature at the time of irradiation (*29*). Tolerance to local kidney X-ray irradiation in the Wistar rat increases with age at time of irradiation (*11*). Shielding of the kidneys in rats at 7–8 weeks of age during TBI with 10.0 Gy X rays prevents kidney injury and increases in risk factors for cardiac disease (*12*). In the current study, unexpected changes in bicarbonate, calcium and potassium suggest a need for further study. Taken together, these findings indicate the importance of the kidney in the mechanism that underlies radiation injury to the heart, and the need to examine the extent that age at time of irradiation links radiation nephropathy to cardiac injury.

Our findings indicate that clinically accepted end points for increased risk of cardiac disease from low-LET radiation at doses as low as 1.5 Gy are not increased within the framework of the current study, and that effects at higher doses (generally 8.0 Gy and above) are age-dependent. The response to γ rays over the dose range 1.5–6.0 Gy was investigated. While we cannot exclude the possibility that a dose below 1.5 Gy could have caused a biological response, we consider this possibility unlikely. Another potential limitation is the duration of the follow-up period. The 270-day follow-up period selected for study was over twice the length used in our previously published studies (*7, 12, 30*) and considered of sufficient duration to allow changes to be observed in the phenotypes measured. However, we did not observe a change in risk factors for cardiac disease or kidney injury in rats irradiated with γ rays over this period for animals exposed to up to 6.0 Gy of photons. Budgetary constraints prevented a follow-up period beyond 270 days.

The duration of future studies may need to be extended beyond 270 days to detect changes in the cardiac and renal end points measured at lower photon doses and such studies will also require larger cohorts to adjust for natural losses associated with an aging cohort. Our findings with low-LET radiation may be useful in studies of the cardiovascular effects of exposure to high-LET radiation. While older, ''astronaut-aged'' rats (6 months old at time of irradiation) did show changes in cardiac risk factors and kidney function after irradiation with sparsely ionizing X- or γ rays, these changes were only evident after a long follow-up period and were generally of lesser magnitude than in younger rats. The absence of an effect on injury after γ-ray irradiation at doses as high as 6.0 Gy, coupled with a delayed and attenuated injury after X-ray irradiation of older rats at higher doses that greatly exceed the dose levels relevant to space flight, suggests that determination of relative biological effectiveness may need to rely on the X-ray results obtained herein.

In summary, this study demonstrates dose-dependent effects of TBI with sparsely ionizing photons on cardiac risk factors in male rats. Strong modifying effects of age at time of irradiation on cardiac and kidney end points relevant to cardiac risk were documented, along with the earlier appearance of key indicators of cardiac risk in animals irradiated at the younger age. The results obtained here will be useful when considering the cardiovascular risks for patients receiving TBI for therapeutic purposes as well as for individuals who may sustain occupational exposures including astronauts and airline crews, and other individuals receiving total-body exposures from radiation accidents or terrorist activities.

SUPPLEMENTARY INFORMATION

Fig. S1. Risk factors for cardiac disease after irradiation of rats at 6 months of age with γ rays. Total cholesterol, HDL cholesterol and triglycerides were essentially unchanged over the 270-day follow period for rats irradiated with 1.5, 3.0 or 6.0 Gy γ rays compared with age-matched sham-irradiated controls.

Fig. S2. Morphological changes to heart of rats at 6 months of age with 6.0 Gy of γ rays. Heart sections were stained with trichrome 270 days after the start of the study.

Fig. S3. Kidney injury after irradiation of rats at 6 months of age with γ rays. BUN, creatinine, total protein and albumin 270 days after irradiation with 1.5, 3.0 or 6.0 Gy.

Fig. S4. Morphological changes to kidney after irradiation of rats at 6 months of age with 6.0 Gy of γ rays. Cortex and medulla of kidney stained with trichrome 270 days after the start of the study.

Fig. S5. Systemic blood pressure after irradiation of rats at 6 months of age with γ rays. Systolic, mean and diastolic pressure 180 and 210 days after irradiation of rats with 1.5, 3.0 or 6.0 Gy γ rays.

APPENDIX A

Table A1. Linear model (without random effects) fitted to various end points.

TABLE A1
Linear Model (without Random Effects) Fitted to Various End Points

	P value for age effect[a]	*P* value for dose effect[b]	*P* value of age at exposure x dose[c]	Dose effect (/Gy) (+95% CI)[d]	Dose × age at exposure effect (/Gy/100 days) (central + 95% CI)[d]
Sodium (mmol/l)	<0.0001	0.5424	0.1066	–0.029 (–0.087, 0.030)	–0.075 (–0.165, 0.016)
Potassium (mmol/l)	0.0281	0.4526	0.8952	–0.033 (–0.092, 0.026)	–0.006 (–0.097, 0.085)
Chlorine (mmol/l)	0.0109	0.7282	0.1926	–0.006 (–0.053, 0.041)	–0.048 (–0.121, 0.024)
Bicarbonate (mmol/l)	<0.0001	<0.0001	0.8044	0.085 (0.037, 0.133)	–0.009 (–0.083, 0.064)
Glucose (mg/dl)	0.5866	0.1072	0.0041	0.077 (–0.440, 0.594)	–1.166 (–1.962, –0.370)
BUN (mg/dl)	0.0089	<0.0001	<0.0001	0.927 (0.685, 1.168)	–0.819 (–1.192, –0.446)
Creatinine (mg/dl)	0.0064	<0.0001	0.0439	0.008 (0.004, 0.011)	–0.006 (–0.012, 0.000)
Calcium (mg/dl)	<0.0001	<0.0001	0.0056	–0.022 (–0.031, –0.014)	–0.018 (–0.031, –0.005)
Albumin (g/dl)	0.0015	<0.0001	<0.0001	–0.040 (–0.047, –0.033)	0.032 (0.021, 0.042)
Protein (g/dl)	<0.0001	<0.0001	<0.0001	–0.060 (–0.069, –0.052)	0.032 (0.019, 0.044)
AST (IU/I)	<0.0001	<0.0001	0.0015	–0.749 (–1.331, –0.167)	1.462 (0.564, 2.361)
Alkaline phosphatase (IU/I)	<0.0001	<0.0001	<0.0001	–1.750 (–2.427, –1.073)	2.393 (1.349, 3.438)
ALT (IU/I)	<0.0001	0.0241	0.0171	–0.004 (–0.425, 0.416)	0.602 (0.108, 1.096)
Total cholesterol (mg/dl)	<0.0001	<0.0001	<0.0001	2.945 (2.394, 3.495)	–2.266 (–3.114, –1.419)
HDL cholesterol (mg/dl)	<0.0001	<0.0001	<0.0001	2.809 (2.319, 3.300)	–1.754 (–2.510, –0.999)
Total cholesterol/HDL ratio	0.0597	0.0551	0.0690	–0.002 (–0.003, 0.000)	–0.002 (–0.005, 0.000)
Triglycerides (mg/dl)	<0.0001	0.0013	0.0011	1.007 (–0.078, 2.092)	–2.798 (–4.472, –1.123)

[a] *P* value of improvement in fit of a model with age at blood draw (days) vs. model with constant term only.

[b] *P* value of improvement in fit of a model with age at blood draw (days) and radiation dose (Gy) vs. model with age at blood draw (days) only.

[c] *P* value of improvement in fit of a model with age at blood draw (days), radiation dose (days), centered age at exposure (days), radiation dose x (centered age at exposure) vs. model with age at blood draw (days), centered age at exposure (days) and dose (Gy) only.

[d] Taken from model with age at blood draw (days), radiation dose (Gy), centered age at exposure (days), radiation dose x (centered age at exposure).

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Brian Fish in the conduct of the X-ray irradiations and dosimetry at MCW. We also thank the members of the BNL support facilities led by Peter Guida, Mary-Ann Petry and Adam Rusek, for outstanding assistance with the gamma-ray studies. Support for this work was provided by the National Aeronautical and Space Administration (NASA grant nos. NNX15AD69G and 80NSSC19K0498), the Greater Milwaukee Foundation, the Foundation for Heart Science, and the We Care Fund.

Received: January 22, 2018; accepted: April 18, 2019; published online: May 16, 2019

REFERENCES

1. Ringborg U, Bergqvist D, Brorsson B, Cavallin-Stahl E, Ceberg J, Einhorn N, et al. The Swedish Council on Technology Assessment in Health Care (SBU) systematic overview of radiotherapy for cancer including a prospective survey of radiotherapy practice in Sweden 2001–summary and conclusions. Acta Oncol 2003; 42:357–65.
2. Orth M, Lauber K, Niyazi M, Friedl AA, Li M, Maihofer C, et al. Current concepts in clinical radiation oncology. Radiat Environ Biophys 2014; 53:1–29.
3. Thomas ED. Kettering Award Lecture. The role of marrow transplantation in the eradication of malignant disease. Cancer 1982; 49:1963–9.
4. Friedman DN, Hilden P, Moskowitz CS, Suzuki M, Boulad F, Kernan NA, et al. Cardiovascular risk factors in survivors of childhood hematopoietic cell transplantation treated with total body irradiation: a longitudinal analysis. Biol Blood Marrow Transplant 2017; 23:475–82.
5. Armenian SH, Sun CL, Vase T, Ness KK, Blum E, Francisco L, et al. Cardiovascular risk factors in hematopoietic cell transplantation survivors: role in development of subsequent cardiovascular disease. Blood 2012; 120:4505–12.
6. Chow EJ, Baker KS, Lee SJ, Flowers ME, Cushing-Haugen KL, Inamoto Y, et al. Influence of conventional cardiovascular risk factors and lifestyle characteristics on cardiovascular disease after hematopoietic cell transplantation. J Clin Oncol 2013; 32:191–98.
7. Baker JE, Fish BL, Su J, Haworth ST, Strande JL, Komorowski RA, et al. 10 Gy total body irradiation increases risk of coronary sclerosis, degeneration of heart structure and function in a rat model. Int J Radiat Biol 2009; 85:1089–100.
8. Shimizu Y, Kodama K, Nishi N, Kasagi F, Suyama A, Soda M, et al. Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950–2003. BMJ 2010; 340, b5349.
9. Little MP, Azizova TV, Bazyka D, Bouffler SD, Cardis E, Chekin S, et al. Systematic review and meta-analysis of circulatory disease from exposure to low-level ionizing radiation and estimates of potential population mortality risks. Environ Health Perspect 2012; 120:1503–11.
10. Little MP. Radiation and circulatory disease. Mutat Res 2016; 770:299–318.
11. Moulder JE, Fish BL. Age dependence of radiation nephropathy in the rat. Radiat Res 1997; 147:349–53.
12. Lenarczyk M, Lam V, Jensen E, Fish BL, Su J, Koprowski S, et al. Cardiac injury after 10 Gy total body irradiation: indirect role of effects on abdominal organs. Radiat Res 2013; 180:247–58.
13. Moulder JE, Cohen EP, Fish BL. Mitigation of experimental radiation nephropathy by renin-equivalent doses of angiotensin converting enzyme inhibitors. Int J Radiat Biol 2014; 90:762–8.
14. Kumar GL, Kiernan JA. Education guide-special stains and H & E: Pathology. 2nd ed. Carpinteria, CA: Dako North America; 2010.
15. Zhang R, Ghosh SN, Zhu D, North PE, Fish BL, Morrow NV, et al. Structural and functional alterations in the rat lung following whole thoracic irradiation with moderate doses: injury and recovery. Int J Radiat Biol 2008; 84:487–97.
16. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika 1965; 52:591–611.
17. Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Statist 1947; 18:50–60.
18. R Package 'lme4′: version 1.1-17. Comprehensive R Archive Network (CRAN). Vienna: R Foundation for Statistical Computing; 2018.
19. R Project version 3.4.4. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2018. (https://www.r-project.org)
20. Straume T, Rugel G, Marchetti A, Rühm W, Korschinek G, McAninch J, et al. Measuring fast neutrons in Hiroshima at distances relevant to atomic-bomb survivors. Nature 2003; 424:539–42.
21. Straume T, Rugel G, Marchetti A, Rühm W, Korschinek G, McAninch J, et al. Addendum: Measuring fast neutrons in Hiroshima at distances relevant to atomic-bomb survivors. Nature 2004; 430:483–83.
22. Sources and effects of ionizing radiation. Vol. I: Sources. UNSCEAR 2000 Report. New York: United Nations; 2000.
23. Sources and effects of ionizing radiation. Vol. II: Effects. UNSCEAR 2000 Report. New York: United Nations; 2000.
24. King V, Constine LS, Clark D, Schwartz RG, Muhs AG, Henzler M, et al. Symptomatic coronary artery disease after mantle irradiation for Hodgkin's disease. Int J Radiat Oncol Biol Phys 1996; 36:881–9.
25. Lehmann R, Bhargava A, Gunzel P. Serum lipoprotein pattern in rats, dogs and monkeys, including method comparison and influence of menstrual cycle in monkeys. Clin Chem Lab Med 1993; 31:633–38.
26. Moulder JE, Fish BL, Cohen EP. Impact of angiotensin II type 2 receptor blockade on experimental radiation nephropathy. Radiat Res 2004; 161:312–7.
27. Adams MJ, Grant EJ, Kodama K, Shimizu Y, Kasagi F, Suyama A, et al. Radiation dose associated with renal failure mortality: a potential pathway to partially explain increased cardiovascular disease mortality observed after whole-body irradiation. Radiat Res 2012; 177:220–8.
28. Sera N, Hida A, Imaizumi M, Nakashima E, Akahoshi M. The association between chronic kidney disease and cardiovascular disease risk factors in atomic bomb survivors. Radiat Res 2013; 179:46–52.
29. Provoost AP, de Keijzer MH, Wolff ED, Molenaar JC. Development of renal function in the rat. The measurement of GFR and ERPF and correlation to body and kidney weight. Ren Physiol 1983; 6:1–9.
30. Lenarczyk M SJ, Haworth ST, Komorowski R, Fish BL, Migrino RQ, Harmann L, et al. Simvastatin mitigates increases in risk factors for and the occurrence of cardiac disease following 10 Gy total body irradiation. Pharmacol Res Perspect 2015; 3:e00145.