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Effects of Radiation on Blood Pressure and Body Weight in the Spontaneously Hypertensive Rat Model. Are Radiation Effects on Blood Pressure Affected by Genetic Background?

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In this work, we utilized spontaneously hypertensive rats (SHR) and Wister Kyoto rats (WKY), from which the SHR was established, to evaluate the effects of whole-body acute radiation on the cardiovascular system at doses from 0 to 4 Gy. In the irradiated SHR, the systolic blood pressure (SBP) increased with increasing dose, while body weight gain decreased with increasing radiation dose. Furthermore, pathological observations of SHR demonstrated that the number of rats with cystic degeneration in the liver increased with increasing dose. The effects observed among SHR, such as increased SBP and retardation of body weight gain, appear very similar to those observed in Japanese atomic bomb survivors. In contrast, the SBP among WKY did not change relative to dose; the body weight, however, did change, as in the SHR. Therefore, the association between radiation exposure and SBP, but not between radiation exposure and retardation of body weight gain, may be affected by genetic background, as evident from strain difference. These results suggest that the SHR and WKY animal models may be useful for studying radiation effects on non-cancer diseases including circulatory diseases, chronic liver disease and developmental retardation.

INTRODUCTION

Epidemiological studies of atomic bomb (A-bomb) survivors, such as the Adult Health Study (AHS) and the Life Span Study (LSS) of the Radiation Effects Research Foundation (RERF), revealed a significant relationship between radiation exposure and circulatory diseases, including stroke, heart failure and myocardial infarction (*1–4*). It is noteworthy that the results of the AHS demonstrated a small but statistically significant positive effect of ionizing radiation on the longitudinal trends of systolic blood pressure (SBP) (*5*). In addition, both body weight and body mass index (BMI) of AHS participants decreased with increasing dose (*6, 7*). However, there are concerns that these phenomena, increased SBP and the retardation in body weight gain and BMI, might be affected by confounding factors such as mental aftermath of A-bomb exposure and socioeconomic problems caused by damages from the A-bomb (*5, 6*). To rule out potential confounding factors and to determine the biological mechanism of radiation effects on blood pressure or body weight gain, an animal model is an essential complementary approach.

Spontaneously hypertensive rats (SHR) established from Wister Kyoto rats (WKY) are widely used as an animal model for human spontaneous hypertension (*8, 9*). In these studies, the WKY is frequently used as control. In the current study, we tested the hypothesis that radiation would exacerbate hypertension in SHR.

MATERIALS AND METHODS

Animals

Specific pathogen-free (SPF) SHR/Izm (SHR) and WKY/Izm (WKY) male rats, 4 weeks old, were purchased from the Disease Model Cooperative Research Association (DMCRA; Kyoto, Japan) through Japan SLC, Inc. (Hamamatsu, Japan). DMCRA conducted appearance inspection of health, body weight, age and sex of rats before the shipment. We also monitored the condition of the animals for one week prior to irradiation. The rats were housed individually in plastic cages [41-cm width (W) × 26-cm length (L) × 22-cm height (H)] on a 12:12 h light-dark schedule. They were fed a normal diet (MF cubed food for mice and rats; Oriental Yeast Co. Ltd., Tokyo, Japan) *ad libitum* without additional salts. Whole-body irradiation

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(WBI) of the rats was performed using a commercial irradiator (Gammacell® 40 Exactor; Best Theratronics, Ottawa, Canada) with cesium-137 source. All rats were non-anesthetized and kept in small plastic boxes [13.5 cm (W) × 9.0 cm (L) × 5.0 cm (H)] to prevent changes in position during irradiation. Irradiations were performed when animals reached 5 weeks of age, at a dose rate of approximately 0.8 Gy/min. To deliver 4 Gy, the rats were irradiated for 5 min. To avoid bias caused by stress, after irradiation the rats were kept in the irradiation equipment for the same time as the irradiation took place (5 min in the 4 Gy experiment). For example, in the case of 2 Gy irradiation, the rats were kept in the machine for additional 2.5 min after a 2.5 min exposure, since the rats irradiated with 4 Gy were kept in the machine for 5 min. The nonirradiated control rats were also kept in the machine for 5 min without exposure. The radiation doses received by the rats in the plastic chambers were verified using glass photoluminescence dosimeters (GD-302M; AGC Techno Glass Co. Ltd, Shizuoka, Japan), which were attached to the surface of the four-sided wall of the boxes. At the start of the SHR study, the number of rats irradiated with 1, 2, 4 and 0 Gy control consisted of 10 rats per dose group. However, one rat each in the 0 Gy and 2 Gy irradiated group died accidentally during blood pressure measurement. Thus, the 1, 2, 4 and 0 Gy irradiated groups consisted of 10, 9, 10 and 9 rats, respectively. The number of WKY irradiated with 2, 4 and 0 Gy consisted of 5 rats each. All rats were kept in the Animal Center of the Research Institute for Radiation Biology and Medicine, Hiroshima University (Hiroshima, Japan), in accordance with the rules and regulations of the Institutional Animal Care and Use Committee.

In this study, we evaluated three end points: 1. blood pressure; 2. body weight; and 3. pathological phenotypes. SHR were randomly allocated into four equally sized groups (1, 2, 4 Gy and 0 Gy as controls) and WKY rats were randomly allocated into three equally sized groups (2, 4 Gy and 0 Gy). All of the above end points were measured in a blind fashion. Each end point was examined as follows.

Measurement of Blood Pressure

Each radiation dose group consisted of 10 rats for SHR and 5 rats for WKY, respectively. As described above, in the 2 Gy and nonirradiated SHR groups, one rat per group accidentally died when blood pressure was measured at 6 weeks postirradiation. Blood pressure was measured one day after irradiation using a tail cuff system by a non-invasive sphygmomanometer (BP-98A-L; Softron, Tokyo, Japan) and then once a week over 30 weeks. All measurements were taken according to the manual from the distributor. In the analyses, we used the mean of four measurements after excluding extreme values (the two highest and two lowest measurements from the total of eight measurements).

Measurements of Body Weight, Food Consumption and Body Composition

Body weight and blood pressure of each rat were simultaneously measured once per week until the rats were sacrificed at 30 weeks postirradiation. The amount of food consumed by each SHR was estimated by measuring the quantity of the food remaining in the tray. Relationship between food consumption and body weight was estimated from the weekly measurements. Body composition was analyzed using an impedance method (ImpediVET®; Bioresearch Co. Ltd., Nagoya, Japan) under anesthesia [optimum amount (40–50 mg/kg) of pentobarbital for each rat] at 20 weeks postirradiation.

Autopsies

Sacrifice was conducted by drawing the blood under anesthesia conditions as described above. A complete necropsy was performed as soon as the rats were sacrificed according to a standard protocol. Terminal body weight, organ weights and any observed gross abnormalities were recorded. Sections from the brain, spleen, heart, liver, kidney, thymus, lung and aorta were collected for histopathological examinations. For the SHR, all organs and tissues were fixed in 10% neutral buffered formalin and shipped from RERF to the Institute for Environmental Sciences (IES; Rokkasho, Japan). The tissue slices from a paraffin-embedded sample were prepared and stained with hematoxylin and eosin (H&E) following standard protocol of the Department of Radiobiology, IES. One H&E slide was prepared and analyzed from each organ of each rat. The lesions were scored by counting the individual number of SHR with the same type of pathological alteration. For the cystic degeneration in liver, we counted the number of SHR whose slide showed the degeneration. All histopathology slides were examined blindly and independently by at least two IES pathologists.

Statistical Methods

Statistical analyses to evaluate associations between radiation exposure and the longitudinal changes of body weight and blood pressure were based on linear mixed effects models, taking into account the correlation within measurements from the same rat (*10*). The model of longitudinal trend of each response (body weight or blood pressure) was selected among linear, linear-quadratic, linear-quadratic-cubic and linear-quadratic-cubic-quartic models based on Akaike information criterion (AIC) and Bayesian information criterion (BIC) (*10*). The equation used for statistical analysis is described in the Appendix. To evaluate the difference of longitudinal changes of each end point across dose groups, interaction terms between the time of irradiation and time since the irradiation were investigated. The time-varying effects of radiation on SBP and body weight were estimated, expressed as change per 1 Gy, and confidence intervals were obtained (*11*). The confidence interval of the time-varying coefficient of radiation exposure was used to establish when the effect of radiation became statistically significant (*11, 12*).

Data on pathological examinations were analyzed by logistic regression to obtain the odds ratio for the prevalence of lesions in each organ examined. Associations between radiation exposure and organ weights were tested, while the appropriateness of a parametric assumption for each organ measurement was evaluated. Tests for trend included the Cochran-Armitage trend test (*13*) or model-based continuous trend in dose. All reported P values were based on two-sided tests. Statistical modeling and testing utilized R version 3.6.0 (http://cran.r-project.org/) and SAS software version 9.3 (Cary, NC).

RESULTS

Blood Pressure

As shown in Fig. 1A, the SBP changes in SHR were followed from 0 to 30 weeks postirradiation, and the change of SBP levels varied by radiation dose group ($P < 0.001$). The significant difference in SBP between irradiated and nonirradiated SHR was not observed within 10 weeks postirradiation. However, the SBP levels of irradiated rats increased relative to nonirradiated rats from 12 to 30 weeks postirradiation. When the effects per 1 Gy were estimated in a regression model, there was an increasing trend for SBP with dose among rats after approximately 12 weeks postirradiation (17 weeks of age) (Fig. 2A). Moreover, after approximately 16 weeks postirradiation, as shown in Fig. 2A, the level of SBP of irradiated SHR was significantly higher than that of the controls.

Conversely, as shown in Fig. 1B, SBP levels of 2 and 4 Gy irradiated WKY were not different from those of the

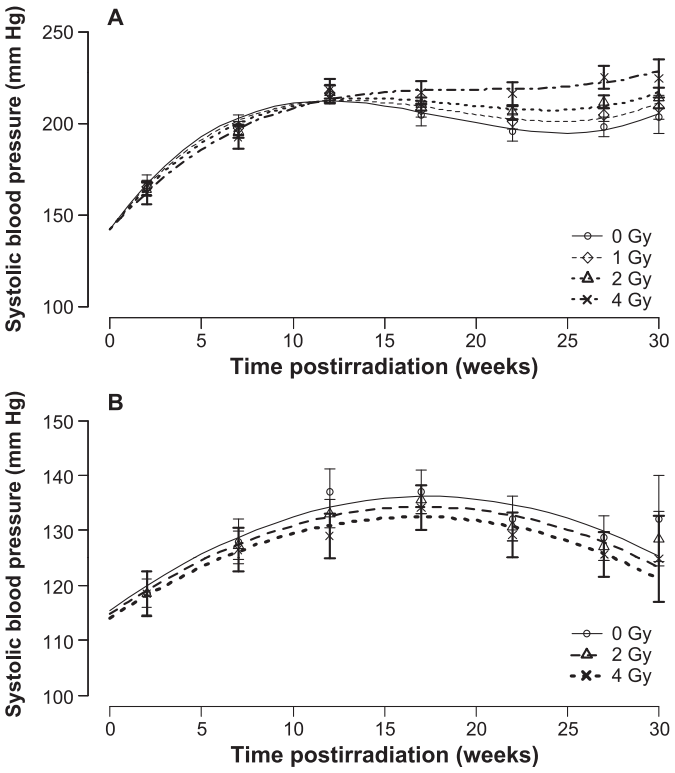


FIG. 1. SBP changes of irradiated SHR and WKY. Panel A: SHR irradiated with 0, 1, 2 and 4 Gy. Statistical analyses were performed according to Eq. (A1). The radiation dose-by-time interaction in a linear mixed effects model was statistically significant ($P < 0.001$). Panel B: WKY irradiated with 0, 2 and 4 Gy. Statistical analyses were similar to those described for panel A. The radiation effect was not significant.

nonirradiated control group, even after 16 weeks postirradiation.

Body Weight

There were no differences in body weight among irradiated and nonirradiated rats before or immediately after irradiation. As shown in Fig. 3, body weights of both the SHR and WKY irradiated with 4 Gy became gradually lower than those of the nonirradiated rats from one week postirradiation. The body weights in 2 Gy irradiated rats also became lower than those of the nonirradiated rats from approximately 10 weeks postirradiation. The differences in body weight persisted through week 30 postirradiation. As shown in the growth curve (Fig. 3), the body weight significantly increased with time, and this trend significantly varied by dose group ($P < 0.001$). The effect per 1 Gy radiation exposure on body weight in the SHR was also estimated using a regression model. As shown in Fig. 2B, a significant, albeit slight difference, was observed as early as one day after irradiation. The body weight pattern among 0, 2 and 4 Gy irradiated WKY showed the same trends as those among the SHR. Statistical analyses of a radiation dose-by-time interaction revealed that those differences were statistically significant ($P < 0.001$) (Fig. 3B).

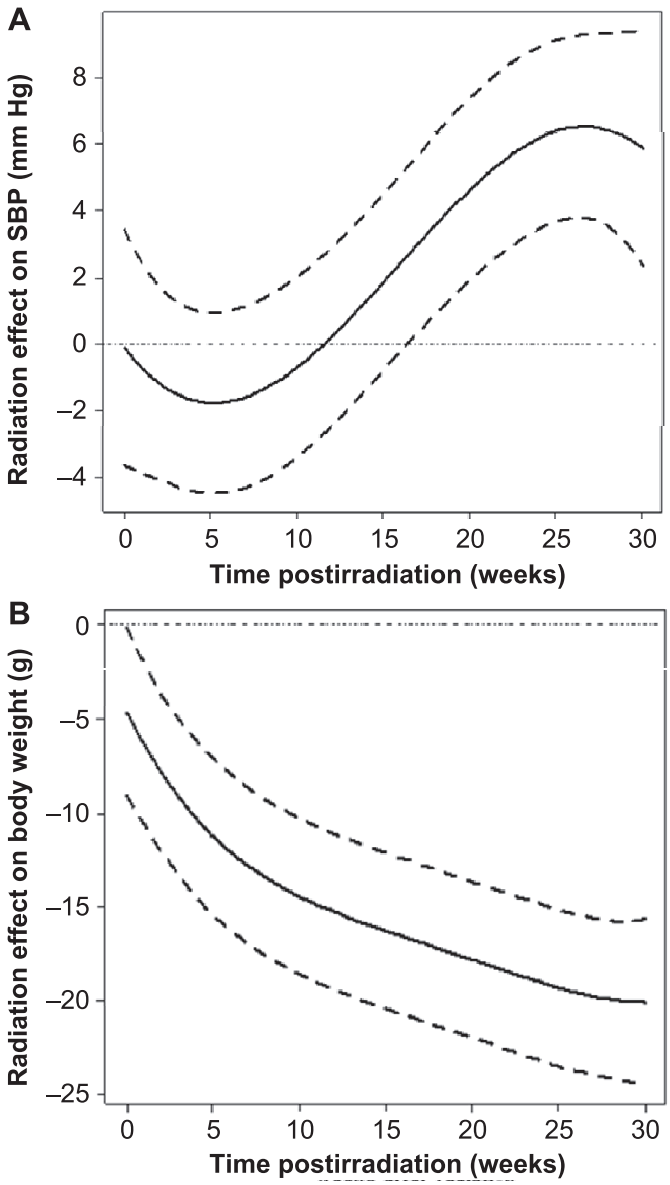


FIG. 2. Effect of 1 Gy radiation on longitudinal change of SBP (panel A) and body weight (panel B) in SHR was determined using the estimated regression model. Each time-trend of SBP change and body weight loss per 1 Gy exposure (mm Hg/Gy and g/Gy, respectively) are shown (solid line) with upper and lower 95% confidence intervals (dashed lines).

To evaluate the cause of the disturbance of body growth and decreased weight in the irradiated SHR, we measured the amount of food consumption (from 5 to 21 weeks of age: 0 to 16 weeks postirradiation) and number of excrements (from 17 to 27 weeks of age: 12 to 22 weeks postirradiation) in both 4 Gy and nonirradiated rats. For each end point, there was no significant difference between the two groups (data not shown). These data support a conclusion that body weight differences after radiation exposure might not be caused by radiation damage to the feeding center or the alimentary system.

Terminal tissue weights, measured at autopsy, were significantly decreased for the thymus ($P < 0.007$), liver

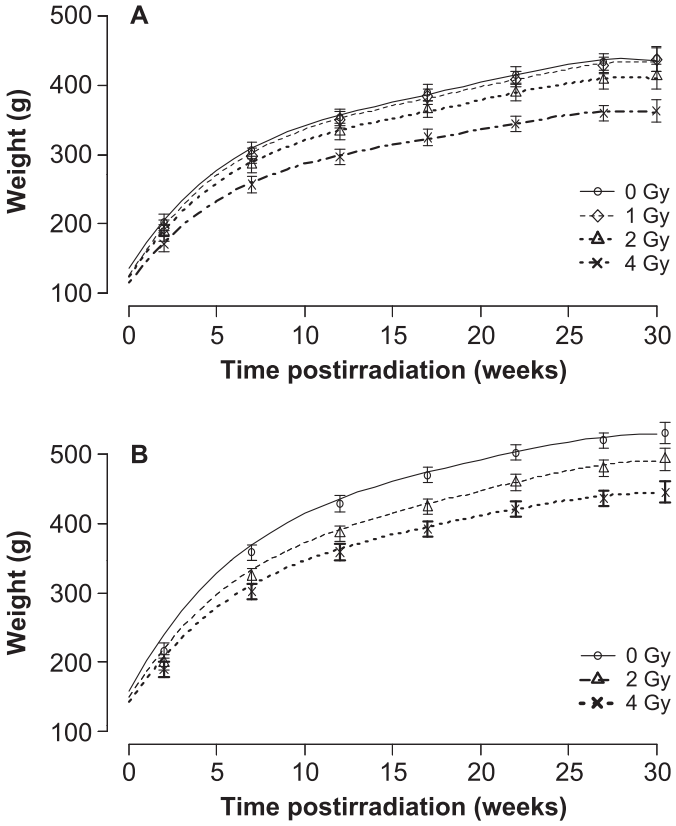


FIG. 3. Body weight changes of SHR (panel A) and WKY (panel B). Statistical analyses were performed according to Eq. (A2). The radiation dose-by-time interaction in a linear mixed effects model was statistically significant ($P < 0.001$) in both SHR and WKY.

($P < 0.002$) and kidney ($P < 0.002$), among the irradiated SHR; the decrease in tissue weights for these organs was well fitted with a linear model (Fig. 4A). By contrast, terminal tissue weights of brain, heart, lung and spleen at autopsy did not change significantly among the irradiated rats (data not shown). Among the WKY, the terminal tissue weights of the thymus ($P < 0.007$) and kidney ($P < 0.002$) were significantly decreased similar to the SHR, but that of the liver was not (Fig. 4B).

The Lesions of Liver

Pathological examinations among the SHR were conducted for various organs, including the brain, kidney, spleen, lung and heart, and no differences related to radiation dose were found. However, the number of SHR showing cystic degeneration in liver (Fig. 5) increased significantly with increasing dose based on a trend test ($P = 0.02$) (Fig. 6), although the dose group-specific prevalence of cystic degeneration in the liver was not statistically different from that in the reference group (0 Gy). By comparison, cystic degeneration was not observed in the liver of the irradiated WKY.[2]

[2] K. Koizumi (personal communication; data not shown).

DISCUSSION

The results of this study using SHR as an animal model suggest that radiation exposure may be associated with the risk and onset of hypertension. This may explain epidemiological findings in the study of the health effects among Japanese A-bomb survivors that found a small, but statistically significant longitudinal trend of increasing blood pressure with increasing ionizing radiation dose. To the best of our knowledge, our work reported here is the first animal study of acute whole-body radiation exposure to demonstrate an association between blood pressure and radiation exposure. In only one other published study, in which the SHR and WKY received partial irradiation to the chest, was blood pressure examined; however this was done only at three days postirradiation (*14*). Our study demonstrates that a new animal model system for evaluating, albeit partly, the association between radiation exposure and development of circulatory disease could be established. This model system can potentially provide important insights into the possible mechanisms underlying the association between radiation exposure and the development of circulatory disease.

As shown in Fig. 1A, SBP levels in SHR irradiated with 1, 2 and 4 Gy were higher than those of 0 Gy irradiated rats beginning at approximately 12 to 30 weeks postirradiation. In A-bomb survivors, Sasaki *et al*. (*5*) used the cubic function of age to illustrate the mixed effects of aging and radiation on SBP. In the AHS study, radiation effect on SBP varied across birth cohorts with subjects born in 1930 or later (age of 15 years or less at time of bombing) showing slightly higher SBP than those among nonexposed subjects with similar age at time of bombing to exposed group, after they were 25 years of age [see Fig. 3 in ref. (*5*)]. In contrast, for cohorts born before 1930 (age 16 years or more at time of bombing), there was an opposite trend: SBP levels among the nonexposed subjects were slightly higher than those among exposed subjects. More recent data reported by Yamada *et al*. (*3*) are consistent with the longitudinal blood pressure trends observed among younger survivors. In the current study, only young (5-week-old) male rats were irradiated. According to the most commonly used method of lifespan comparison, one might assume that 5-week-old rats are equivalent to 7-to-10-year-old human children. The radiation effect on SBP was observed after 15 weeks of age, which is equivalent to 20-to-30-year-old human adults. Thus, the results observed in our SHR model appear to have similar tendency to those found in the A-bomb survivors (5, *15*).

It is believed that psychological stress after the atomic bombing might have influenced SBP in the survivors. In a published psychological study conducted among the AHS population (*15*), it was reported that the frequency of complaints related to health problems and psychosomatic symptoms increased proportionally with radiation dose. This finding suggests that the experience of the atomic

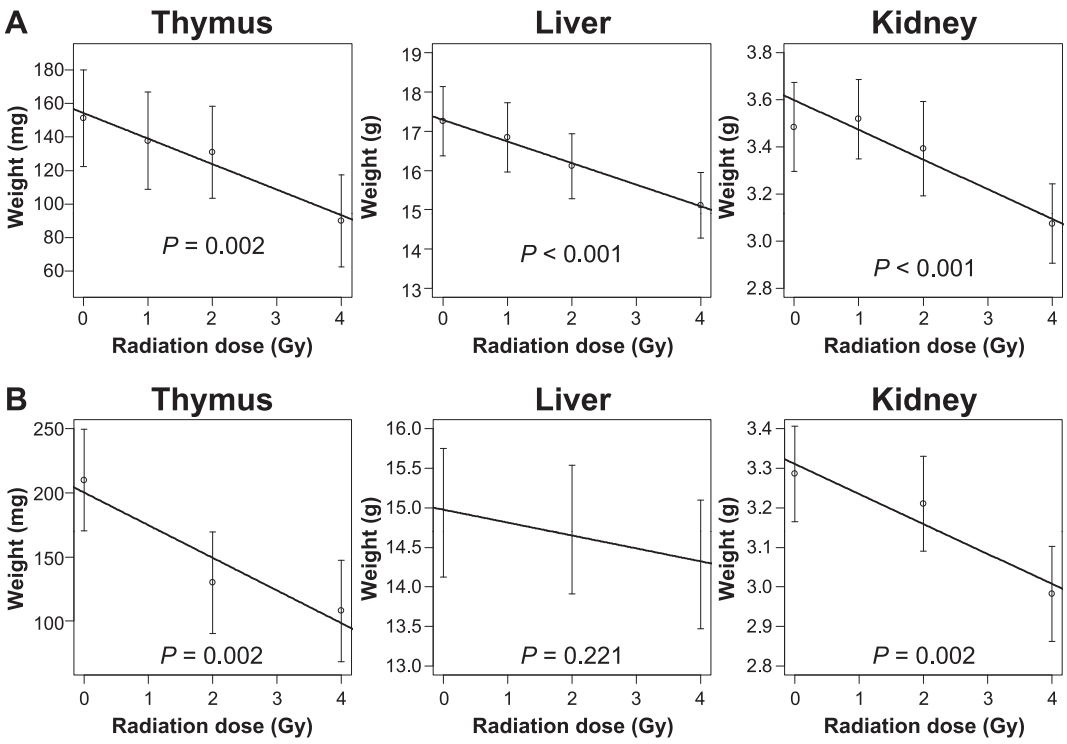


FIG. 4. Association between terminal tissue weight of SHR (panel A) and WKY (panel B) and radiation dose. Linear-dose-response relationships are demonstrated for each organ with 95% confidence intervals. Trend *P* values are described in each panel.

bomb catastrophe affected the survivors' mental health. The emotional consequences of nuclear power station disasters have been reported after the Three Mile Island and Chernobyl accidents (*16*). In contrast to Yamada's report (*15*), emotional consequences after nuclear power station disasters were unrelated to radiation dose. These effects are often long-term and associated with fears of developing both cancer and non-cancer diseases. Published studies of Estonian Chernobyl cleanup workers (*17*) demonstrated higher-than-expected rates of psychosomatic symptoms among early entrants to the disaster zone. Published preliminary data (*16*) from Fukushima disaster-affected populations also suggest that mothers of young children and workers are at risk of experiencing psychosomatic symptoms both as a direct result of radiation fear and an indirect result of societal stigma.

As described above, there are many published studies in which increased blood pressure was reported after psychological stress via activation of sympathetic nerve function (*18, 19*). In our rat study, we attempted, to the best of our abilities, to minimize the confounding effect of psychological stress on blood pressure, by exposing all animals to equal and minimal amounts of stress; nevertheless, SBP increased with increasing dose in SHR. Thus, our finding suggests that radiation exposure increases blood pressure as a late effect.

As for the inherited nature of essential hypertension, this has been developed in many rat models, including SHR, Dahl salt-sensitive (s) and salt-resistant (r) rats (*20*). In these animal models, many quantitative trait loci (QTLs) associated with blood pressure were found, and genome-wide sequencing studies began elucidating the nature of QTLs. In their published work, Padmanabhan and Joe (*21*) have pointed out that there are many coding genes associated with blood pressure, but at the same time there are many more noncoding elements in the genome, which might operate on blood pressure through epistatic or gene-gene interactions. In addition, genomic elements appear to control the diversity of symbiotic bacterial flora in the gut and bacterial products such as short-chain fatty acids; these in turn might control blood pressure in the host (*22*). Thus, radiation might affect blood pressure by altering the expression or function of coding and non-coding genes associated with blood pressure, as well as through modification of microbiota-host interactions.

Although it remains unknown what mechanisms are involved in the increase of SBP after radiation exposure, our data demonstrated that SHR and WKY are useful animal models to study mechanisms of radiation effects on the circulatory system, including effects on vascular biology/disease, blood pressure-related genomic alterations and perivascular inflammation, which are relevant to A-bomb survivors.

In addition to the association between radiation dose and SBP level, we observed an association between radiation dose and retardation of body weight gain. As shown in Fig.

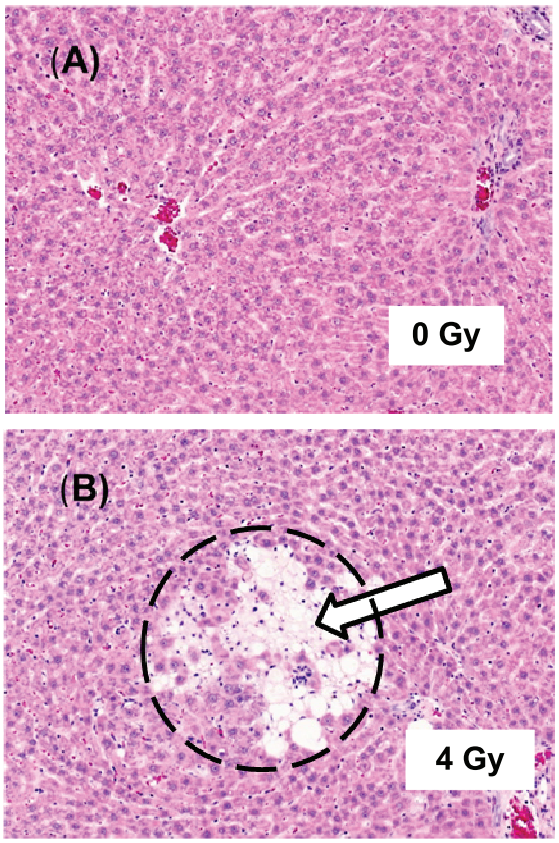
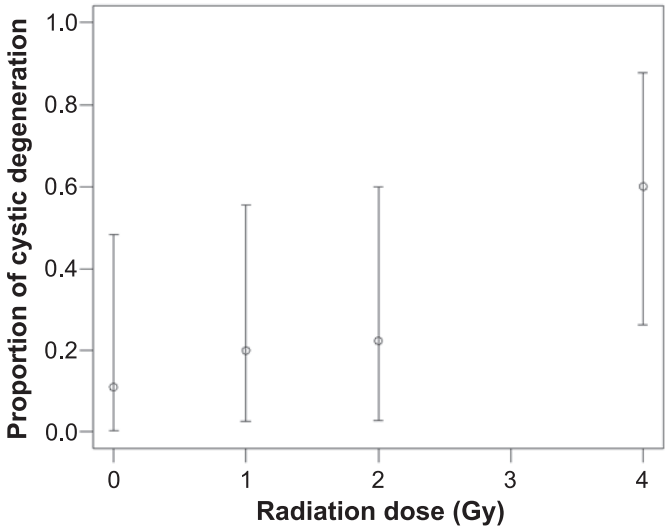


FIG. 5. Pathological examinations (H&E stained) of SHR livers. Samples were collected from the rats at 30 weeks postirradiation. Cystic degeneration was observed in panel B, but not in panel A, as indicated by the circle and arrow.



	Nonirradiated	1 Gy	2 Gy	4 Gy
Cystic degeneration observed/examined rats	1/9	2/10	2/9	6/10

FIG. 6. Results of cystic degeneration observed in liver. The individual numbers of rats showing cystic degeneration were counted and the proportion was calculated for each dose group. The confidence intervals were calculated as an exact method using R binom package. The 95% confidence intervals were overlaid on each of the circles showing the upper and lower bounds with horizontal short line segments.

3, the difference in body weight among the rats irradiated with 2 Gy, 4 Gy and the 0 Gy controls became larger over time. The body weight gain retardation in irradiated SHR was observed as a statistically significant change according to radiation dose by time interaction ($P < 0.001$), and the value of fat mass index of 4 Gy irradiated rats was smaller than that of nonirradiated rats (data not shown). As several organs of irradiated rats were smaller than organs of nonirradiated control rats at necropsy (Fig. 4), and no tumors were found at necropsy (data not shown), we assume that the retardation of body weight gain may be causally related to radiation exposure and, at least in part, due to growth retardation of the whole-body including tissues and organs. Otake *et al*. (*6*) reported a linear effect of A-bomb radiation on the reduction of growth among survivors younger than 10 years old at the time of bombing. Otake *et al*. (*6*) also evaluated the possible contribution of poor nutrition and disruption of normal family life in the years immediately after the war from various data. They concluded that nutrition and socioeconomic status affected all individuals studied equally, regardless of radiation dose received. Our results also support that poor nutrition was not the main cause for dose-dependent decrease in body weight, since, as noted before, our animals consumed equal amounts of food.

Unexpectedly, we also found that radiation increased cystic degeneration in the liver of SHR (Figs. 5 and 6) but not in WKY. Cystic degeneration develops from liver stellate (Ito) cells in rats, and is rarely observed in other mammals after administering genotoxic and non-genotoxic compounds, and is associated with stellate cell activation and proliferation (*23*). It has been proposed that hepatic injury of any etiology might result in stellate cell activation, proliferation and fibrosis in other species (*24*). The AHS studies reported a significantly increased incidence of chronic liver disease and cirrhosis with radiation dose, and those findings are consistent with the LSS findings (*25*). In Japan, the predominant causes of chronic hepatitis and cirrhosis are HCV or HBV infection and excessive alcohol intake (*26*). The dose-related increase in the incidence of chronic liver disease and cirrhosis in the AHS study might be partially explained by a persistent HBV infection or acceleration of active HCV infection among the heavily exposed survivors. However, it appears that cirrhosis without hepatitis virus infection comprises most of the dose-associated chronic liver disease (*27*). The dose response for cystic degeneration observed among SHR in our studies suggests that the significantly increased incidence of liver damage with radiation dose was caused by true radiation exposure, since the rats were not affected by HCV or HBV infection or excessive alcohol intake.

Although the mechanisms for elevated SBP and body weight gain reduction with radiation dose are still unclear, phenotypes (blood pressure, body weight, organ weight and cystic degeneration in the liver) examined in this study appear to be caused by, or closely related to, exposure to radiation. This study represents a promising approach for investigating radiation effects on the accelerated onset of circulatory disease and circulatory system-related diseases, such as hypertension, fatty liver diseases and diabetes. We consider our findings as early steps towards improved mechanistic understanding of the relationship between radiation exposure and development of circulatory and lifestyle-related diseases. Further studies are needed, including those of vascular biology and pathology, as well as new omics studies, to elucidate the mechanisms of radiation effects on changes in blood pressure and body weight.

Male rats were used exclusively in this study, since it is well known that female sex hormones frequently cause bias in studies involving the circulatory system. However, future studies using female rats may provide particularly interesting results, since the data obtained from female atomic bomb survivors were different from that of male survivors [e.g. (*28*)].

CONCLUSION

The data in this SHR study demonstrated that the increase in SBP and weight gain retardation observed among A-bomb survivors could be caused directly by radiation. The data showing a divergence in tissue response to radiation among two rat strains provided useful information about disease progression after radiation exposure.

APPENDIX

The longitudinal trajectories of SBP and body weight were modeled, respectively, for the *j*th measurement of *i*th subject as:

$$SBP_{ij} = \beta_0 + b_i + \beta_1 \mathrm{age}_{ij} + \beta_2 \mathrm{age}_{ij}^2 + \beta_3 \mathrm{age}_{ij}^3 + \gamma_1 dose + \gamma_2 dose \cdot \mathrm{age}_{ij} + \gamma_3 dose \cdot \mathrm{age}_{ij}^2 + \gamma_3 dose \cdot \beta_2 \mathrm{age}_{ij}^3 + \varepsilon_{ij} \quad \text{(A1)}$$

and

$$weight_{ij} = \alpha_0 + b_i + \alpha_1 \mathrm{age}_{ij} + \alpha_2 \mathrm{age}_{ij}^2 + \alpha_3 \mathrm{age}_{ij}^3 + \alpha_4 \mathrm{age}_{ij}^4 + \gamma_{w1} dose + \gamma_{w2} dose \cdot \mathrm{age}_{ij} + \gamma_{w3} dose \cdot \mathrm{age}_{ij}^2 + \gamma_{w4} dose \cdot \mathrm{age}_{ij}^3 + \gamma_{w5} dose \cdot \mathrm{age}_{ij}^4 + \varepsilon_{ij}, \quad i = 1, \ldots, n,\ j = 1, \ldots, J, \quad \text{(A2)}$$

where α, β and γ are parameters to be estimated and the parameter estimates were obtained as shown in Tables A1–A4, b_i is a random intercept, or subject-specific intercept with $b_i \sim N(0, \sigma_b^2)$, and ε_{ij} and δ_{ij} are random noise with $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ and $\delta_{ij} \sim N(0, \sigma_\delta^2)$, respectively. The evaluation of longitudinal change due to radiation exposure was based on likelihood ratio test with 3 degrees of freedom for SBP model and 4 degrees of freedom for weight model, respectively. Also, the linear-quadratic model of weeks after irradiation was the best fit blood pressure of WKY based on AIC and BIC and the respective figures shown are based on the best fit model.

Table A1
Fixed Effect Parameter Estimates of SBP for SHR

	Parameter	95% Confidence intervals	*P* value
Intercept[a]	209.8	(203.9, 215.8)	<0.0001
Dose	1.859	(–0.684, 4.402)	0.150
Age (centered at 20)	–1.483	(–1.957, –1.008)	<0.0001
Age[b]	–0.159	(–0.190, –0.128)	<0.0001
Age[c]	0.016	(0.013, 0.019)	<0.0001
Dose × age	0.569	(0.365, 0.773)	<0.0001
Dose × age[b]	0.005	(–0.009, 0.018)	0.510
Dose × age[c]	–0.002	(–0.003, –0.001)	0.003

[a] SBP of control at age 20 weeks, or 15 weeks postirradiation.

Table A2
Fixed Effect Parameter Estimates of SBP for WKY

	Parameter	95% Confidence intervals	*P* value
Intercept[a]	135.8	(131.6, 140.0)	<0.0001
Dose	–0.881	(–2.512, 0.750)	0.309
Age (centered at 20)	0.329	(0.115, 0.542)	0.003
Age[b]	–0.068	(–0.094, –0.043)	<0.0001
Dose × age	0.001	(–0.106, 0.060)	0.587
Dose × age[b]	0.005	(–0.009, 0.011)	0.869

[a] SBP of control at age 20 weeks, or 15 weeks postirradiation.

Table A3
Fixed Effect Parameter Estimates of Weight for SHR

	Parameter	95% Confidence intervals	*P* value
Intercept[a]	381.6	(372.9, 390.3)	<0.0001
Dose	–16.247	(–19.975, –12.519)	<0.0001
Age (centered at 20)	5.868	(5.492, 6.244)	<0.0001
Age[b]	–0.089	(–0.125, –0.052)	<0.0001
Age[c]	0.020	(0.019, 0.022)	<0.0001
Age[d]	–0.001	(–0.002, –0.001)	<0.0001
Dose × age	–0.315	(–0.477, –0.154)	<0.0001
Dose × age[b]	0.004	(–0.011, 0.020)	0.597
Dose × age[c]	–0.001	(–0.002, –0.0002)	0.010
Dose × age[d]	0.0001	(0.00004, 0.0002)	0.003

[a] Weight of control at age 20 weeks, or 15 weeks postirradiation.

Table A4
Fixed Effect Parameter Estimates of Weight for WKY

	Parameter	95% Confidence intervals	*P* value
Intercept[a]	458.1	(449.2, 467.0)	<0.0001
Dose	–19.184	(–22.621, –15.746)	<0.0001
Age (centered at 20)	7.359	(6.931, 7.786)	<0.0001
Age[b]	–0.249	(–0.290, –0.209)	<0.0001
Age[c]	0.022	(0.021, 0.024)	<0.0001
Age[d]	–0.001	(–0.001, –0.001)	<0.0001
Dose × age	–0.312	(–0.478, –0.146)	0.0002
Dose × age[b]	0.024	(0.008, 0.039)	0.003
Dose × age[c]	–0.001	(–0.002, –0.0005)	0.001
Dose × age[d]	0.00002	(–0.00004, 0.0001)	0.427

[a] Weight of control at age 20 weeks, or 15 weeks postirradiation.

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