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Irradiation of Varying Volumes of Rat Lung to Same Mean Lung Dose: a Little to a Lot or a Lot to a Little?

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Abstract

Purpose
To investigate whether irradiating small lung volumes with a large dose or irradiating large lung volumes with a small dose, given the same mean lung dose (MLD), has a different effect on pulmonary function in laboratory animals.

Methods and Materials
WAG/Rij/MCW male rats were exposed to single fractions of 300 kVp X-rays. Four treatments, in decreasing order of irradiated lung volume, were administered: (1) whole lung irradiation, (2) right lung irradiation, (3) left lung irradiation, and (4) irradiation of a small lung volume with four narrow beams. The irradiation times were chosen to accumulate the same MLD of 10, 12.5, or 15 Gy with each irradiated lung volume. The development of radiation-induced lung injury for ≤20 weeks was evaluated as increased breathing frequency, mortality, and histopathologic changes in the irradiated and control rats.

Results
A significant elevation of respiratory rate, which correlated with the lung volume exposed to single small doses (≥5 Gy), but not with the MLD, was observed. The survival of the rats in the whole-lung-irradiated group was MLD dependent, with all events occurring between 4.5 and 9 weeks after irradiation. No mortality was observed in the partial-volume irradiated rats.

Conclusions
The lung volume irradiated to small doses might be the dominant factor influencing the loss of pulmonary function in the rat model of radiation-induced lung injury. Caution should be used when new radiotherapy techniques that result in irradiation of large volumes of normal tissue are used for the treatment of lung cancer and other tumors in the thorax.

Keywords
Radiation-induced pulmonary injury, Rat lung, Mean lung dose, Lung intensity-modulated radiotherapy

Introduction
Radiation-induced lung complications, such as radiation pneumonitis (RP) and pulmonary fibrosis, is the most common factors limiting the radiation dose that can be delivered to thoracic tumors. The risk of pulmonary complications increases with both the absorbed dose and the irradiated volume. Complex three-dimensional dose distributions within the healthy lung are often reduced to simple metrics, such as the mean lung dose (MLD) or the lung volume receiving a threshold dose of ≥x Gy (Vx), to facilitate analysis of the outcomes and evaluation of treatment plans (1). The MLD and V20 often emerge as the best prognostic factors of RP incidence in
clinical studies\(^2,3,4\), suggesting that a reduction in the dose deposition in all volumes of the lung provides the best strategy for preventing pulmonary complications. However, some investigators\(^3,5,6,7,8\) observed that RP risk correlates more strongly with the low-dose region of a dose–volume histogram (DVH). Thus, the \(V_X\) corresponding to a threshold of 5–13 Gy has been found to be the most significant dosimetric factor predicting the incidence of RP in several recent studies\(^3,5,6,7\). Gopal et al.\(^8\) showed that a significant loss of lung function expressed as a reduction in the diffusing capacity for carbon monoxide occurs at a relatively low dose of 13 Gy.

The possibility that irradiation of a large lung volume with doses as low as 5 Gy might lead to a high incidence of RP poses a potential problem for the increasing use of intensity-modulated radiotherapy (IMRT) techniques in the treatment of thoracic tumors. These techniques tend to use multiple low-intensity beams to concentrate the dose to the target at the expense of irradiating larger volumes of normal tissue with small doses. As a result, IMRT plans for the treatment of lung tumors are generally characterized by increased values of \(V_5\) compared with three-dimensional conformal RT (3D-CRT) plans. For example, Murshed et al.\(^9\) created nine-field IMRT plans for 41 non–small-cell lung cancer (NSCLC) patients who had previously undergone 3D-CRT. These investigators reported that, although the MLD and \(V_{20}\) were reduced on average by 2 Gy and 10%, respectively, in the IMRT plans compared with the 3D-CRT plans, \(V_{10}\), and, especially \(V_5\), were harder to control. Specifically, \(V_5\) was increased in 63% of all cases. Cannon et al.\(^10\) have compared various DVH metrics among patients treated with 3D-CRT, traditional IMRT, and helical IMRT (tomotherapy). No statistically significant differences were found in the MLD among the IMRT and 3D-CRT plans, albeit the MLD tended to be smaller for the IMRT patients. The \(V_5\) was 20% greater in the traditional IMRT plans and 25% greater in the helical IMRT plans, and the \(V_{10}\) was 15% greater in all IMRT plans compared with the 3D-CRT plans, the differences being statistically significant.

In contrast to studies that have emphasized the importance of low-dose volumes, other investigators observed the most significant correlations of RP risk with greater dose volumes, such as \(V_{30}\). Willner et al.\(^12\) reported that an increase in \(V_{40}\) resulted in the greatest increase in the RP rate and concluded that it is reasonable to disperse the dose over large areas of the lung to reduce the volumes receiving >40 Gy. The question of which treatment approach (i.e., “a lot to a little” or “a little to a lot”) results in lower lung complication risk remains without a definitive answer. Theoretical studies\(^13\) have been equally inconclusive because different normal tissue complication probability models offer opposite answers to this problem.

The aim of this study was to investigate, using an animal model, how radiation-induced pulmonary function loss depends on the irradiated lung volumes for the same MLD.

**Methods and Materials**

**Animals and experimental design**

Male WAG/Rij/MCW rats were maintained in the animal care facility of the Medical College of Wisconsin, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The rats were housed 3 to a cage with a 12-h light/12-h dark cycle and fed sterilized rat chow (Harlan Teklad, Madison, WI) and water \textit{ad libitum}.

Four treatments, in decreasing order of irradiated lung volume, were administered: (1) whole lung irradiation, (2) right lung irradiation, (3) left lung irradiation, and (4) irradiation of a partial (~20%) volume of both lungs. In the latter group, four discrete regions approximately 5 mm in diameter were exposed, one in the apex and base of both the left and the right lungs. Because evidence from other animal studies has shown that the basal lung might be more radiosensitive than the apical portion\(^14,15\) and vice versa\(^16\), irradiation ports exposing regions in both lower and upper lung were used to minimize this effect. The beam-on times were such
that the accumulated MLD was the same for each group, resulting in longer irradiation times when smaller volumes of lung were exposed. For each irradiated lung volume, the rats were subdivided into three groups receiving a MLD of 10, 12.5, or 15 Gy in a single fraction. Whole-lung doses in this range have been demonstrated to produce a differential response with regard to the induction of DNA damage in the rat lung, yet not to cause significant lethality (15). Four animals per dose level per irradiated volume were used. The control group consisted of four age-matched, nonirradiated animals. The rats \((n = 52)\) were 7–9 weeks old (median 8.1) and weighed 130–180 g (median, 157) at irradiation. The Institutional Animal Care and Use Committee of the Medical College of Wisconsin reviewed and approved all procedures.

Dosimetry

Before irradiation, free-breathing thorax scans were performed in 4 rats using a microfocal X-ray imaging system designed and constructed at the Zablocki Veterans Affairs Medical Center/Keck Functional Imaging Center (Milwaukee, WI). Details of the imaging system and procedures have been previously published (17). The rats were anesthetized (82 mg/kg ketamine and 1.8 mg/kg acepromazine intramuscularly) and placed upright in a custom-designed positioning jig. The thoracic regions were scanned in a step-and-shoot mode, with 1° increments to acquire 360 X-ray images. The X-ray tube was operated at 30 kV and 265 μA. The 3D image volumes were reconstructed with isotropic resolution of ∼100 μm/pixel using a Feldkamp cone beam reconstruction algorithm.

The lungs and heart were semiautomatically segmented in ImageJ, version 1.36b (National Institutes of Health, Bethesda, MD). Coronal lung projections from the 4 scanned rats were superimposed, and the resulting image was used to design Cerrobend blocks to confine exposure to specific regions of the lung in each of the four irradiated volume groups. The positioning jig was filled with a polymethyl methacrylate phantom and secured in the irradiation position. The dose distributions at the entrance and exit surfaces of the jig were measured with each of the four Cerrobend shields using Gafchromic EBT film (International Specialty Products, Wayne, NJ). The exposed films were scanned using a Vidar VXR-12 digitizer (VIDAR Systems, Herndon, VA), and the images were calibrated using RIT113, version 4.4, film dosimetry software (Radiological Imaging Technology, Colorado Springs, CO). Absolute dose maps were then imported into MATLAB R2006b (MathWorks, Natick, MA) and registered with coronal computed tomography images of the 4 animals (Fig. 1). The thorax entrance and exit dose was 13.7 and 8.2 Gy in the whole-lung group, 23.2 and 13.4 Gy in the right-lung group, 29.2 and 16.3 Gy in the left-lung group, and 52 and 27 Gy in the four-region group, respectively. The relative depth doses were measured in the polymethyl methacrylate phantom placed inside the jig using a PinPoint ionization chamber (PTW N31006, 0.015 cm³) and were found to decrease linearly \((R^2 > 0.998)\) for all shielding configurations. The dose absorbed in each lung or heart voxel was calculated by linear interpolation between doses determined by projecting the voxel onto the entrance and exit dose maps. The obtained dose distributions were used to calculate the mean doses and construct DVHs (Fig. 2). The average DVHs of the 4 scanned animals (Fig. 2a) were used to calculate the MLD associated with each irradiated lung volume. The irradiation times were chosen so that the same MLD of 10, 12.5, or 15 Gy would be accumulated with each of the four exposure configurations. Because the scanned rats were of the same strain and sex and similar in size to the irradiated animals, the calculated doses should represent the doses received by the rats during irradiation.
Irradiation

In preparation for irradiation, the rats were anesthetized with an isoflurane/oxygen mixture (1% isoflurane) and placed in the same jig and vertical position used for computed tomography imaging. A single radiation fraction was administered through the rats’ dorsal surface using a Pantak HF320 X-ray machine (East Haven, CT) operated at 300 kV and 10.5 mA, with a 0.1-mm Cu/2.5-mm Al filter (1.4 mm Cu half-value layer). The rats were
monitored with a Webcam to ensure that they were properly positioned and immobilized during the entire irradiation period. The dose rate at the midline of the rat was 2.3 Gy/min.

Breathing rate measurements
The loss of lung function was determined using biweekly measurements of breathing frequency in the irradiated rats and nonirradiated controls. The rats were placed in an airtight chamber, and the pressure inside the chamber was recorded for 2–3 min in each rat. Four uniform, disjoint samples, each 10–12 s in duration, were used to count the breaths. The samples were combined, and the counts were converted into breaths per minute. Baseline breathing rates were measured 1 or 2 days before irradiation. The changes in breathing rates were followed for 20 weeks.

Excised lung imaging
At the end of the 20-week noninvasive observation period, the lungs were excised and imaged in 5 rats (1 rat for each irradiated volume and 1 control rat chosen randomly within their respective groups). All irradiated rats were from the 10-Gy MLD group. Details of the excised lung imaging protocol have been previously described [17]. During perfusion of the isolated lungs, the flow rate was varied from 5 to 30 mL/min and the pulmonary artery pressure recorded. X-ray images were acquired at selected angles. The X-ray tube was operated at 24 kV and 334 μA. A salt solution in the pulmonary arteries was then replaced with perfluorooctyl bromide for X-ray contrast, and the image acquisition sequence was repeated.

Histologic examination
The rats were killed by an isoflurane overdose when they had become moribund or after 20 weeks. The period of tissue collection from the rats that survived to the end of the 20-week noninvasive study period was 20.1–22.7 weeks (median, 20.7). The lungs and heart were dissected, fixed in 10% buffered formaldehyde for 24 h, and embedded in paraffin. Four-micron sections were cut, stained with hematoxylin-eosin, and examined under a BX41 light microscope (Olympus America, Center Valley, PA).

Statistical analysis
Mixed linear models with the autoregressive error structure were used to account for repeated measures in the respiratory rate data. The differences in baseline respiration rates were assessed using one-way analysis of variance. The Kaplan-Meier method was used to estimate survival probabilities, and the log–rank test was used to determine significance between dose groups. All analyses were performed using Statistical Analysis Systems, version 9.1 (SAS Institute, Cary, NC) with the significance level set at 0.05.

Results
Dose distributions in rat lung
Dose distributions within the lung, which were estimated using computed tomography images of the 4 representative animals, are shown in Fig. 2a. These DVHs correspond to the 10-Gy MLD; in the 12.5- and 15-Gy groups, the doses shown on the horizontal axis would scale proportionally to the MLD. The vertical axis was normalized to a total lung volume of each rat. The average total lung volume of the 4 animals was 4.48 ± 0.23 cm³ (mean ± standard error), corresponding to a coefficient of variation of 10%. An approximately linear relationship was found between an animal’s weight and the total lung volume (data not shown). Although larger rats tended to have greater lung volumes, no discernable trend was found between the MLD and animal weight. The mean dose and standard error for the whole, right, and left lung-irradiated animals was 10 ± 0.08, 10 ± 0.05, and 10 ± 0.06 Gy, respectively, resulting in coefficients of variation of 1.5%, 1.0%, and 1.2%. The MLD was more variable in the small-volume group (i.e., 10 ± 0.60 Gy), corresponding to a coefficient of variation of 12%. This trend was also seen in the greater spread of DVH curves for individual rats in this treatment group (Fig. 2a).
These observations indicated that, despite the variations in the lung volume with animal weight, a deviation of the MLD received by individual rats from its nominal value was minimal in the treatment groups involving irradiation of significant lung volumes. A larger spread of the MLD in the four-region group reflected difficulties in precisely irradiating partial organ volumes in small animals.

Radiation-induced morbidity and mortality
Most rats developed skin lesions in the exposed areas 2–4 weeks after irradiation. The lesions were successfully controlled by topical application of Animax. Five rats died suddenly, and 4 moribund rats exhibiting a hunched posture, labored breathing, and cyanosis were euthanized before the end of the 20-week study period. All prematurely dead rats were from the whole lung-irradiated group. Postmortem examinations revealed pleuropericardial effusions and cardiomegaly. Figure 3 shows the survival curves for the whole lung-irradiated animals, stratified by the MLD. All deaths occurred 4.5–9 weeks after irradiation. A marked dose response was found, with the rats that had had their whole lung exposed to a lower MLD surviving longer. The mean survival time was 5 weeks in the 15-Gy group and 7 weeks in the 12.5-Gy group. In the 10-Gy group, 1 rat died at 6 weeks, and the remaining 3 survived to 20 weeks. The survival curves were significantly different ($p = 0.015$).

Breathing rate elevation
Figure 4 shows the breathing rates as a function of time elapsed after irradiation. Marked increases in the respiratory rates were observed in the rats that had undergone unilateral (right or left lung) and bilateral (whole lung) irradiation. A small elevation of breathing rates was also seen in the small volume-irradiated rats at ≥16 weeks. The data from each irradiated volume group were first analyzed separately to determine the presence of a dose effect. Mixed linear models (with the time point and MLD treated as fixed factors and the rat treated as a random factor) showed that the effect of the follow-up point was highly significant ($p < 0.0001$) and that of the MLD was not significant ($p ≥ 0.43$) in all treatment groups. Because the whole lung-irradiated group contained censored data, only the breathing rates measured before the first death had occurred (i.e., measurements at Weeks 0, 2, and 4) were entered into the analysis. A model used to analyze the control breathing rates contained the follow-up point as a sole fixed effect, which was found to be not significant ($p = 0.97$). This result indicates that no trend toward changes in the breathing rates was present among the nonirradiated rats over time and suggests that the breathing rate elevation in the irradiated rats resulted from radiation injury rather than ageing and/or environmental factors.
In Fig. 4, the breathing rates for different MLD levels were pooled together within the respective irradiated volume groups. A first-order polynomial was fit to the data from each treatment group. In the bilaterally irradiated group, only the breathing rates recorded ≤4 weeks (before censoring occurred) were used for the fitting. Because the baseline breathing rates were not significantly different \((p = 0.23)\) among all 13 (four irradiated volume groups multiplied by the three dose levels plus the controls) groups, the overall average baseline breathing rate of 176 breaths/min was used as a common intercept for the linear fits. The slopes of the fitted lines represent an average increase in the respiratory rate per week of time after irradiation and could be regarded as a surrogate of severity of pulmonary injury after radiation exposure. The slopes appeared to increase with increasing volume of irradiated lung tissue. To further emphasize this point, in Fig. 6, the weekly breathing rate increase was plotted as a function of lung volume irradiated to doses ≥5 Gy (reference line in Fig. 2a). This result suggests that, for the same range of MLD, the severity of radiation-induced pulmonary injury is strongly related to the lung volume exposed to low radiation doses.

In Fig. 5, the breathing rates for different MLD levels were pooled together within the respective irradiated volume groups. A first-order polynomial was fit to the data from each treatment group. In the bilaterally irradiated group, only the breathing rates recorded ≤4 weeks (before censoring occurred) were used for the fitting. Because the baseline breathing rates were not significantly different \((p = 0.23)\) among all 13 (four irradiated volume groups multiplied by the three dose levels plus the controls) groups, the overall average baseline breathing rate of 176 breaths/min was used as a common intercept for the linear fits. The slopes of the fitted lines represent an average increase in the respiratory rate per week of time after irradiation and could be regarded as a surrogate of severity of pulmonary injury after radiation exposure. The slopes appeared to increase with increasing volume of irradiated lung tissue. To further emphasize this point, in Fig. 6, the weekly breathing rate increase was plotted as a function of lung volume irradiated to doses ≥5 Gy (reference line in Fig. 2a). This result suggests that, for the same range of MLD, the severity of radiation-induced pulmonary injury is strongly related to the lung volume exposed to low radiation doses.

**Fig. 4.** Average breathing frequency as function of time after irradiation for different irradiated volume groups and mean lung dose levels. Error bars represent standard error of mean. Same control data reproduced on each panel for comparison with data from irradiated rats. Bpm = breaths per minute.

**Fig. 5.** Average breathing frequency in different irradiated volume groups as function of time after irradiation. Error bars represent standard error of mean. Legend shows linear fit equation for each group. bpm = breaths per minute; BR = breathing rate.
Excised lung imaging

Figure 7 shows images of the excised perfused lungs of randomly chosen rats from each of the four irradiated volume groups (10 Gy MLD) and the control group. The lungs that had undergone bilateral exposure (Fig. 7b) appeared to be significantly denser than the lungs of the control rats (Fig. 7a), probably because of the development of fibrosis\(^\text{18}\). Small spots of high density found outside the vasculature (indicated by arrows) were confirmed to be calcifications on histopathologic analysis. In the right and left lung-irradiated rats (Fig. 7c,d), significant shrinkage and remodeling of the ipsilateral lung was seen. The lungs of the rat with the smallest lung volume irradiated (Fig. 7e) had visible regions of greater density in the areas in which the X-ray beam passed through the lung tissue.

The hemodynamic data from the isolated lung study are shown in Fig. 8. The rat that had undergone bilateral lung irradiation exhibited pulmonary hypertension. Unilaterally irradiated rats, despite significant damage to the ipsilateral lung, had relatively low pulmonary vascular resistance. Similar to the breathing rate data, pulmonary vascular resistance appeared to increase as the lung volume exposed to low radiation doses (≥5 Gy) increases. However, this observation was based on the examination of lungs of a single rat from each irradiated volume group and, therefore, should be interpreted with caution.
Fig. 8. Pressure-flow plots obtained in isolated perfused lungs from unirradiated rat and rats with different lung volumes exposed to same mean lung dose of 10 Gy.

Histopathologic changes
Significant parenchymal and vascular changes were observed in the irradiated areas of the lung of all treated rats compared with the control rats. In the whole lung-irradiated group (Fig. 9), common findings included pulmonary artery damage, diffuse alveolar damage, and fibrosis. The severity of damage generally increased as the MLD increased. In the 12.5-Gy group, large areas (~50% of tissue) showed endothelial detachment of blood vessels, epithelial detachment of bronchi, significant necrosis, and fibrosis of parenchyma. In the 15-Gy group, most tissue (>75%) was affected by diffuse necrosis and degenerative changes of lung parenchyma, bronchiole, and blood vessels. The remaining areas showed diffuse interstitial fibrosis. No significant areas of necrosis were found in the 10-Gy irradiated rats that survived to 20 weeks. On the basis of these findings, the premature deaths in the high-dose (12.5 and 15 Gy), bilaterally irradiated rats were most likely caused by respiratory failure, resulting from the massive and severe lung damage.

Fig. 9. Histologic changes in lungs of bilaterally irradiated rats compared with control rats (400×). Control and 10-Gy samples collected at 21 weeks after irradiation. Samples from 12.5- and 15-Gy groups collected when moribund rats were euthanized at 6–9 and 5–6 weeks after irradiation, respectively.

Figure 10 shows representative views of lung tissues from the bilaterally, unilaterally, and small volume-irradiated rats (10 Gy MLD) compared with those from the control rats. The tissues of bilaterally and unilaterally irradiated rats showed diffuse consolidation, and tissues of small volume-irradiated rats showed patchy consolidation that coincided with the exposed areas. Although consolidation appeared to increase with the
MLD, no clear effect of dose on the severity of damage was seen for the other endpoints in the unilaterally and small volume-irradiated rats. The predominant findings in the bilaterally and unilaterally irradiated rats were diffuse alveolar damage and fibrosis. Generally, no difference was found in the histopathologic findings between right and left lung irradiation. The predominant pathologic findings in the areas of lung irradiated with four narrow beams was acute alveolar damage and edema. Pulmonary artery damage, focal hemorrhage, and hemosiderin deposition, indicating old hemorrhages was observed in all irradiated rats.

![Image](image-url)

**Fig. 10.** Histologic changes at 21 weeks after irradiation in bilaterally, unilaterally, and small volume-irradiated rats compared with control rats (400×). Irradiated rats received mean lung dose of 10 Gy. (A) Pulmonary artery damage, (B) diffuse alveolar damage and fibrosis, and (C) acute alveolar damage and edema. Inserts show relative degree of tissue consolidation after each treatment (20×).

The examination of heart sections showed significant changes in bilaterally irradiated rats compared with the control rats. The findings included focal hemorrhage, focal fibrosis, chronic inflammatory infiltrate, and myocardial damage with cytoplasmic vacuole formation (data not shown). The damage was more severe in the prematurely dead rats exposed to the MLD of 12.5 and 15 Gy than in the surviving 10-Gy irradiated rats. The cardiac histopathologic changes in the other groups of rats exposed to irradiation were relatively minor compared with those in the whole lung-irradiated group.

**Discussion**

Although the DVH metrics, such as the MLD or Vx, contain partial information about both the dose absorbed in an organ and the irradiated volume, it is commonly recognized that these quantities cannot reflect the entire complexity of the dose–volume relationships (19). In the present study, we observed some MLD dependence of irradiation-related mortality (Fig. 3) and histopathologic changes (Fig. 9) among the whole lung-irradiated rats. However, no clear trend was found in respiratory rate elevation as a function of MLD (Fig. 4). In contrast, our results suggest that the volume of the whole lung irradiated to small doses might be the most significant prognostic factor for radiation-induced lung injury (Fig. 6, Fig. 8). The quantity plotted in Fig. 6 is not equivalent to the V5 metric used in clinical studies because single-fraction doses were used in our experiments. In contrast, the clinical V5 values usually correspond to 60–66 Gy regimens in ~2-Gy fractions. However, altered fractionation and interspecies differences are expected to have no bearing on the applicability of our qualitative findings to a clinical situation.

Novakova-Jiresova et al. (20) also investigated the dose–volume dependence of respiratory function in a rat model of radiation-induced lung injury. They irradiated 100%, 50%, and 25% of lung volume to uniform doses (using parallel-opposed fields) of 9–12, 16–22, and 27–36 Gy, respectively. These dose–volume combinations...
corresponded to approximate MLD ranges of 9–12, 8–11, and 7–9 Gy for the three amounts of irradiated lung volume. In our study, we opted to irradiate varied lung volumes to the same MLD to provide a clinically relevant common denominator for the dose–volume distribution in a critical organ. Regardless of the differences in experimental design, our findings have corroborated the main conclusion of Novakova-Jiresova et al. (20) that a low dose scattered over a large lung volume causes more early (≤20 weeks after irradiation) toxicity than an extreme dose confined to a small volume.

Several studies have shown that the tolerance dose for pulmonary function loss could be significantly reduced if large portions of the heart lie within the irradiation field 16, 21. To minimize this effect, we irradiated all rats from the dorsal surface rather than with parallel-opposed beams. The mean heart doses corresponding to different irradiated lung volumes (10 Gy MLD) are listed in Fig. 2b. As expected, irradiation of the left lung resulted in the largest mean heart dose of 9.7 Gy. Although the heart received a much lower dose of 6.5 Gy when the right lung was irradiated, the respiratory rates were elevated to a greater degree in the right lung-irradiated group (Fig. 5). This observation implies that the irradiated volume of the lung, and not the heart dose, plays the dominant role in the breathing rate increase. It is also possible that the interaction between lung and heart injury was not observed in this study because the mean heart dose was less (even in the 15-Gy MLD group) than the putative threshold of about 18 Gy, above which heart irradiation has been hypothesized to enhance pulmonary function loss by modulating a functional reserve of the lung 21. Although the confounding effect of cardiac irradiation was likely minimal in the partial volume-irradiated rats in our experiments, the histopathologic findings suggest that heart damage might have partially contributed to the death of the whole lung-irradiated rats.

Our results unequivocally suggest that concentrating the dose in small lung volumes (i.e., “a lot to a little”) provides a better strategy for reducing the risk of lung complications in RT patients. This conclusion highlights a possible problem with the use of multibeam conformal RT techniques, such as IMRT, for the treatment of thoracic tumors. The decrease of MLD with IMRT is accompanied by an increase in the low dose-irradiated volume 9, 10. Because a lower MLD is expected to reduce the risk of RP and a larger low dose-irradiated volume is expected to increase the risk, the overall effect will be determined by a tradeoff between these two trends. In our experiments, even the lowest MLD of 10 Gy resulted in greater elevation of breathing frequency and mortality in the whole lung-irradiated rats than the greatest MLD (15 Gy) delivered to small volumes of the rat lung. This observation, extended to comparisons between IMRT and 3D-CRT dose distributions, implies that the MLD decrease might not be sufficient to overcome the adverse effect of an increase in the low dose-irradiated lung volume. Wang et al. (6) have also hypothesized that the risk of PR depends more strongly on the irradiated volume than on the dose. Combining IMRT and 3D-CRT data in future clinical analyses is expected to result in models for the prediction of lung toxicity that would more prominently feature the effect of irradiated lung volume.

Taking precautions to minimize exposure of large volumes of normal lung, several centers are proceeding with implementation of IMRT for the treatment of NSCLC, and the initial toxicity results have recently emerged. The Memorial Sloan-Kettering Cancer Center investigators (22) observed a greater rate of acute RP in a group of 35 patients treated with IMRT compared with a stage-matched group of 97 3D-CRT patients, although the difference was not statistically significant. In contrast, Yom et al. (23) reported that in advanced NSCLC patients, IMRT resulted in significantly lower levels of Grade 3 or worse RP compared with 3D-CRT, despite significantly larger V5 values for the IMRT patients. A low rate of RP was also observed in a dose-escalation trial for locally advanced and medically inoperable NSCLC using helical tomotherapy 24. The potential hazard of large lung volume irradiation with IMRT warrants additional investigation in the format of clinical trials. One such trial has been initiated at the University of Texas M.D. Anderson Cancer Center (23); its results are eagerly awaited.
Conclusions
Our findings in the whole lung-irradiated rats compared to the rats from the other treatment groups receiving the same MLD include, mortality the greatest elevation of respiratory rates per week of time after irradiation, a tendency toward pulmonary hypertension, and the most severe degenerative changes in the lung tissues. These findings suggest that exposure of a large volume of healthy lung to low radiation doses is the most significant factor influencing pulmonary function loss in rats. Additional preclinical and clinical studies are necessary to determine the dose–volume parameters that correlate most accurately with the risk of RP. Until then, great caution should be exercised when multibeam and rotational delivery RT techniques are used for the treatment of NSCLC and other tumors in the thorax.

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