Pulmonary Radiation Injury: Identification of Risk Factors Associated with Regional Hypersensitivity

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Abstract

Effective radiation treatment of thoracic tumors is often limited by radiosensitivity of surrounding tissues. Several experimental studies have suggested variations in radiosensitivity of different pulmonary regions. Mice and rat studies in part contradict each other and urge for a more detailed analysis. This study was designed to obtain a more comprehensive insight in radiation injury development, expression, and its regional heterogeneity in lung. The latter is obviously highly critical for optimization of radiotherapy treatment plans and may shed light on the mechanisms of lung dysfunction after irradiation. Six different but volume-equivalent regions in rat lung were irradiated. Whereas the severity of damage, as seen in histologic analysis, was comparable in all regions, the degree of lung dysfunction, measured as breathing rates, largely varied. During the pneumonitic phase (early: 6-12 weeks), the most sensitive regions contained a substantial part of alveolar lung parenchyma. Also, a trend for hypersensitivity was observed when the heart lay in the irradiation field. In the fibrotic phase (late: 34-38 weeks), lung parenchyma and heart-encompassing regions were the most sensitive. No impact of the heart was observed during the intermediate phase (16-28 weeks). The severity of respiratory dysfunction after partial thoracic irradiation is likely governed by an interaction between pulmonary and cardiac functional deficits. As a repercussion, more severe acute and delayed toxicity should be expected after combined lung and heart irradiation. This should be considered in the process of radiotherapy treatment planning of thoracic malignancies. (Cancer Res 2005; 65(9): 3568-76)

Introduction

As the most frequent malignant tumors routinely treated by radiation occur in a thoracic region, reducing the risk of radiation injury to adjacent healthy organs is a crucial issue in clinical radiotherapy and radiation biology. The lung is a very radiosensitive, vital organ, damage to which can lead to complications in up to one fifth of the patients undergoing radiotherapy (1-6). The sequelae come typically in two phases: acute inflammatory pneumonitis and late pulmonary fibrosis (3, 4, 7-10). Both pathologic processes (i.e., inflammatory infiltration, congestion, and endothelial damage in the former and replacement of functional parenchyma by connective tissue in the latter) lead to compromised lung perfusion, increased vascular resistance, reduced gas-exchange interphase between air and blood, and suboptimal blood oxygenation. Clinical symptoms range from dyspnoea on effort to respiratory failure, right heart failure, and possibly death (4, 6, 8).

As curative radiotherapy in the thoracic region is generally delivered using external radiation beams, the irradiation of normal tissues is an inevitable side effect of achieving tumoricidal dose within a tumor surrounded by those tissues. Modern treatment planning technology allows comparison between dose distributions of several treatment alternatives in patients before commencing the treatment, and thus provides a margin for clinical decision-making. Therefore, efforts are aimed at creating clinically applicable routine strategies enabling precise treatment tailoring in each individual patient, maximizing the dose delivery to a tumor, yet preventing severe toxicity risks. Such tailoring requires thorough knowledge of factors determining the risk of normal tissue complications.

The role of physical dosimetric factors, such as total radiation dose, irradiated volume (1, 2, 7, 11, 12), and dose per fraction (5, 7), has received considerable attention. Although those factors are clearly relevant, several lines of evidence suggest that they alone are insufficient for an accurate estimation of complication probabilities (13, 14). Therefore, the attention turned towards an investigation of possible biological determinants. Besides inhomogeneous lung perfusion (15, 16) and cytokine effects (17, 18), the location of the irradiated subvolume within the lung is likely to play an important role.

In humans, regional heterogeneity in response to lung irradiation was suggested in a prospective trial of lung cancer patients, where the middle or lower lung location of an irradiated subvolume was related to a higher risk of complications than the upper lung location (1). This, however, was only significant in univariate but not multivariate analysis, likely due to limitations of clinical studies where matching identical irradiated volumes in different locations is a difficult task. In that aspect the main emphasis lies with experimental studies. A pioneering work has been done in mice. Here, the lung base appeared to be the most sensitive in terms of higher morbidity [breathing rate (BR) elevation] and lethality after irradiation of similar subvolumes compared with lung apex or mid-region (19, 20). The authors linked this regionally heterogeneous response to differences in the proportion of gas-exchange structures (alveoli) within the entire organ inherent to the anatomic architecture of tracheobronchial tree. It was proposed that the location of the subvolume along the vertical axis (i.e., the apical, mid-region, or basal location) has to be taken into account when predicting an impact of lung injury (21).

To obtain a more comprehensive insight in radiation injury development, expression, and its dependence on regional heterogeneity in lung, we used a rat model of partial lung irradiation expanded to more clinically relevant regions and allowing higher...
accuracy of dose delivery than previous studies, thanks to the larger size of rat thorax (22). Our preliminary studies confirmed the importance of the location of the irradiated subvolume but, at the same time, yielded first estimates of sensitivity that were partially contradictory to the mice data of the Travis group (22, 23). This required testing an alternative hypothesis that the bronchial tree layout and alveolar density, being similar in rats and mice, might not be the only determinants of the regionally heterogeneous radiation response. Our current study addressed this issue by investigating the outcome of targeted irradiation of 50% subvolumes in the right, left, apical, basal, mediastinal (central), and lateral (peripheral) lung regions. We show differences in radiation response between these regions in terms of the lung function (BR elevation) and show that these differences fluctuate in relation to the time elapsed from irradiation. Using a histopathologic evaluation, we documented that those regional differences are not due to selective radiosensitivity of the regions as such. Rather, the data suggest that alveolar density in the affected lung regions and an additional, external factor of heart irradiation interact over a time and jointly determine the global outcome of the thoracic irradiation in terms of respiratory function.

Materials and Methods

Animals. Adult male albino Wistar rats (n = 232) of the Hsd/Cpb:WU strain bred in a specific pathogen free colony (Harlan-Cpb, Bijnswijk, the Netherlands) were used in the experiments. They were housed five to a cage under a 12-hour light/12-hour dark cycle and fed rodent chow (RMH-B, Hope Farms, Woerden, the Netherlands) and water ad libitum. The experiments were done in agreement with the Netherlands Experiments on Animals Act (1977) and the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Strasbourg, 18.III.1986).

Collimator design. Three-millimeter lead collimators were constructed for anterior-posterior and posterior-anterior irradiations targeted to six different lung regions: right, left, apical, basal, mediastinal, or lateral. Based on thoracic computed tomography (CT) scans of five nonirradiated rats weighing 300 to 340 g, the borders of the collimators were calculated to expose precisely 50% (±5%) of total lung volume (including both the alveolar parenchyma and bronchial structures encompassed within). Details of the procedure have been published earlier (22, 24). Simulator images (anterior-posterior) of resulting irradiation portals are shown in Fig. 1. The alveoli essential for the gas exchange are not homogeneously distributed over the whole lung as certain parts contain more large bronchi (21). Based on literature and the anatomy as observed during the extirpation of rat lungs, it was noted that the mediastinal region, in contrast to the lateral region, contained more large bronchi and blood vessels and, as a result, less functional parenchyma. For the right, apical, and basal regions no differences were observed. CT-based analysis of pulmonary density revealed similar patterns.

The irradiation portals as designed for this study also involved the heart. The volume of the heart in the irradiation fields was for the right region 25 ± 5%, left 75 ± 5%, apical 99 ± 1%, basal 1 ± 1%, mediastinal 97 ± 3%, and for the lateral region 3 ± 3% (see Fig. 1).

Irradiation procedure. Positioning of the animals and dosimetry were adapted from procedures used previously for parotid gland irradiations in this laboratory (24). Rats weighing 280 to 320 g were anaesthetized with xylazine (6 mg kg⁻¹) and ketamine (40 mg kg⁻¹) and positioned in a polymethylmethacrylate holder hanging vertically by their upper incisor teeth fitted in a groove of a positioning rod just behind an appropriate collimator. The hanging position did not influence the density distribution in the lung tissue as determined by CT-density measurements. The thorax outside the irradiation field as well as the rest of the body was shielded by 3-mm lead plate. One of the six lung regions (Fig. 1) was irradiated from two parallel opposing anterior-posterior/posterior-anterior irradiation fields using an orthovoltage X-ray machine (Mueller MG 300, Philips, Eindhoven, the Netherlands) operated at 200 kV and 15 mA (0.5-mm Cu and Al filters, HVL₁ = 1.05 mm Cu). Two separate positioning holders were needed for the anterior-posterior and posterior-anterior irradiations that followed immediately one another. The total doses were delivered within 20 minutes in each animal. At a focus-skin distance of 21.3 cm, the midplane dose rate was determined using the average of the entrance and exit dose rates. The entrance and exit dose rates were determined by means of thermoluminescent dosimetry on live shaved rats and checked against tabulated percentage dose distributions. The difference in collimator size was included in the dose rate using tabulated backscatter factors and the percentage dose distributions. The nominal dose rate was 2.68 to 2.90 Gy min⁻¹. It was verified from the percentage dose distributions and by a radiochromic film (GafChromic, type MD-55, ISP Technologies, Inc., Wayne, New Jersey) exposure in a wax phantom with a rat equivalent diameter of 35 mm that the dose inhomogeneity across the thorax in the beams direction was ≤7%. Using dose profile films, the 15% and 10% isodoses were found to extend not more than 4.5 and 10 mm beyond the edge of the shield, respectively. We assumed those doses

Figure 1. Simulator films of the anterior-posterior irradiation portals for the 50% right (A), left (B), apical (C), basal (D), mediastinal (E), and lateral (F) lung regions. Note the heart contour in the left, apical, and mediastinal fields.
negligible in terms of "in field" effects. "In-beam" monitoring of the X-ray tube output was done during the irradiations using an ionization chamber (PTW "Fibermax," B30001, Freiburg, Germany).

Within each of the six region cohorts (right, left, apical, etc.), four dose groups were irradiated by a single dose either of 16, 18, 20, or 22 Gy. Control animals were anaesthetized and sham irradiated.

Follow-up and morbidity. Two-hundred thirty-two animals were inspected for a minimum of twice a week for general health and weighed biweekly. The irradiation did not lead to major alterations in food intake although the animals from 18 to 22 Gy dose groups lagged mildly behind controls in their weight gain (data not shown). We encountered 13 cases of unplanned sacrifice or death due to anaesthesia overdose (4), sanguineous nasal fothing (2), purulent bronchopneumonia (1), >10% weight loss (1), spinal paralysis (1), and causes unrelated to the experimental procedure (4). We did not observe any morbidity among controls.

The numbers of animals per dose group reduced during the follow-up due to sacrifices for histology: they were 9, 7, and 5 between 0 and 8, 10 and 26, and 28 and 38 weeks, respectively. The control group consisted of 14, 10, and 7 animals in these periods.

Breathing rate assay. BR assay is an established method for evaluation of respiratory function in rodents (25, 26). After two training sessions, a BR assay at rest was recorded for each rat less than a week before the irradiation and then every 2 weeks till 38 weeks postirradiation. As described earlier (22, 27), an unrestrained animal was placed in a 1,500-ml air-tight but transparent tube of a whole-body plethysmograph connected to a pressure transducer. The frequency of pressure changes inside the tube was recorded and displayed on a calibrated chart as breaths per minute (bpm). A mean BR of an animal was then calculated from a minimum of four steady regions of the recording lasting ≥15 seconds. If the measurement required more than 5 minutes to obtain, the animal was let out of the tube and rested to prevent anxiety as well as drop of oxygen inside the tube. A mean BR of a dose group (bpm) with its SE was calculated from the means of individual animals at each time point.

Histology. Two rats per dose group and three to four controls were selected at random and sacrificed by an i.p. pentobarbital overdose at 8, 26, and 38 weeks postirradiation. Whereas the heart was still beating, the animals were heparinized, the thoracic cavity exposed, and pulmonary and systemic circulation perfused with PBS (pH 7.3) under a macroscopically distinguishable forming the expected shape of the irradiation portal. The damaged (presumably irradiated) parts of every specimen were separated; standardized tissue samples were excised from irradiation portal. The damaged (presumably irradiated) parts of every specimen were separated; standardized tissue samples were excised from irradiation portal. The damaged (presumably irradiated) parts of every specimen were separated; standardized tissue samples were excised from irradiation portal.

Three-micrometer serial sections were mounted on slides, stained with H&E or Masson's trichrome stain for collagen fibers (bluish green), and examined by light microscopy. The definition pathology in the irradiated lung parenchyma was a focal lesion distinguished by inflammatory infiltrate, interstitial and/or intra-alveolar edema, and fibrotic obliteration of alveoli (see Fig. 2). The severity of focal lesions on each slide was assessed using a semiquantitative scoring system: no foci present = score 0; small foci present (focus < 1/2 of 100 × magnification field) = score 1; medium foci present (focus ≤ 100 × field) = score 2; large foci present (focus ≤ 40 × field) and total affected area ≤ 50% of the total tissue cross-section = score 3; confluent foci present (focus exceeds 40 × field) and total affected area > 50% of the total tissue cross-section = score 4. One H&E and one Masson's trichrome slide per rat were examined and assigned a joint score.

Statistical analysis. The comparison of BR values among the six region cohorts was done in three distinct periods of BR dynamics: early phase (6-12 weeks), intermediate phase (16-28 weeks), and late phase (34-38 weeks). Within these periods, the BR values of individual animals from 18 to 22 Gy dose groups belonging to one region cohort (Table 1), or to one or two region cohorts (Fig. 3C), were pooled together and compared with the BR values of other cohorts. Because the values were not normally distributed, nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test, the SPSS software package version 11.0) were used. The Mann Whitney U values were adjusted according to the Bonferroni correction for multiple comparisons. The nominal level of statistical significance (after the adjustment) was 5%.

The same tests were used for comparison of histologic scores among the six region cohorts. The scores of six rats from 18 to 22 Gy dose groups of one region cohort sacrificed at one time point (8, 26, or 38 weeks) were pooled together and compared with the scores of other cohorts (Table 1).

Odds ratios (OR) that were used for analysis of BR data were derived from 2 × 2 tables and their 90% confidence intervals (CI) were calculated using the SE of the logarithm of the appropriate OR. The 90% CIs were chosen (rather than 95% CI) to assess the 5% significance only in the lower tail area (i.e., to assess the 5% probability that the OR is not higher than 1).

Results

Pathology and histopathology. The perfused and inflated lung specimens were examined macroscopically on autopsy. At 8 weeks postirradiation, the irradiated regions were darker (hyaeremic) than the shielded regions. They contained small whitish patches apparent through the darkened pleural surface. No obvious shrinkage was visible. However, severe shrinkage of the irradiated regions was observed at the higher doses (≥20 Gy) from 26 weeks onwards (see Supplementary data). Thus, the macroscopic pathology was initially dominated by signs of inflammation (8 weeks) and later by fibrotic retraction (26 and 38 weeks).

At microscopic evaluation, a delicate structure of alveolar tissue was apparent in nonirradiated controls at all time points (Fig. 2A-F). At 8 weeks after irradiation, a dose-dependent increase in inflammatory foci dispersed throughout normally looking parenchyma was observed in the irradiated lungs, ranging from <10% to >50% of the total tissue cross-section on a slide. Within these foci, interstitial and, at higher doses, intra-alveolar oedema (exudate) occurred. The alveolar spaces were filled by inflammatory infiltrate consisting mainly of alveolar macrophages and occasionally a few neutrophils (Fig. 2G and J). A variable degree of collagen deposition (none to moderate) accompanied the inflammatory process (Fig. 2J).

At later times after radiation (26 and 38 weeks), the foci acquired a fibrous character. Dose-dependent buildup of interstitial collagen led from focal septal thickening to complete obliteration of vast areas of alveolar spaces. Where alveoli disappeared, only lumina of bronchi and hypertrophic vessels

Figure 2. Histologic changes in lung parenchyma at 8 weeks (A and D; control; G and J, right lung after 22 Gy), 26 weeks (B and E; control; H and K, left lung after 22 Gy), and 38 weeks (C and F; control; I and L, lateral region after 22 Gy). Serial sections were stained with H&E (upper) and Masson's trichrome stain (lower). In control tissue (A-F), normal parenchyma with thin alveolar septa (1) and collagen deposits (green) limited to the adventitia of bronchi (2) or vessels (3) could be seen at all time points. Findings 8 weeks after irradiation (G and J) revealed an acute exudative inflammation. The alveoli were filled with inflammatory infiltrate (1) and eosin-stained exudate (2). Atypical type II pneumocytes (3) were observed. Alveolar septa were edematous and infiltrated (4). Initial collagen deposition (green) appeared occasionally (5). Chronic productive inflammation dominated at 26 (H and K) and 38 (I and L) weeks. Alveolar spaces (rudiments, 4) were obliterated by a connective tissue with diffuse collagen deposits (green, 5) engulfing lumina of bronchi (2) and hypertrophic vessels (3). Persisting inflammatory infiltrate (1). Time pattern of the changes is shown in the scheme (M).
gaped in compact connective tissue (Fig. 2H, K, I, and L). Mononuclear inflammatory infiltration was still present in remaining alveoli and mainly in the interstitium. The intra-alveolar exudate was rare at 26 weeks and not observed at 38 weeks. Together, these findings indicate resorption of the acute exudative inflammation and its replacement by a chronic interstitial inflammation and fibro-proliferation and progressive collagen accumulation from 26 weeks onwards (Fig. 2M).

Respiratory function. To investigate radiation-induced changes in pulmonary function, BRs were assayed biweekly over 38 weeks postirradiation (Fig. 3). A dose-dependent increase in BR was observed after ≥18 Gy in all cohorts (typical example Fig. 3A). Similar time-dependent changes were observed for all regions (Fig. 3B). The first increase in BR appeared between 6 and 12 weeks, followed by a decrease and a subsequent second increase between 16 and 28 weeks. Beyond 30 weeks, a second recovery occurred. Combining this BR dynamics with histologic data, three phases could be distinguished (Figs. 2M and 3B): an early phase (week 6–12) of an acute exudative inflammation paralleled by the first BR peak, and an intermediate phase (week 16–28) and a late phase (week 34–38), both characterized by a chronic fibro-proliferative inflammation with either a generalized increase in BR (the intermediate phase) or a variable BR recovery (the late phase).

Regional variations. Despite the general similarities in terms of the dynamics of the respiratory response, the magnitude of the response varied between the regions. Table 1 shows the six region cohorts ordered according to the severity of the respiratory dysfunction (represented by the mean bpm) in each of the three phases. This functional rating is compared with the semiquantitative histopathologic score. It seems that morphology failed to explain the clear-cut variations in dysfunction. Rather, these variations seemed to be governed by the character of organ structures contained in the irradiation field. The cohorts that contained 50% of the alveolar tissue and no heart in the irradiation portal (right and basal) behaved similarly but distinctly from the cohorts that contained either >50% proportion of alveolar tissue (lateral) or 50% of the alveolar tissue plus heart in the irradiation field (left and apical). The mediastinal cohort containing <50% proportion of the alveolar tissue but a major proportion of the heart stood usually apart from the other regions. As such, the data of regions containing most of the functional parenchyma (lateral), roughly half of the functional parenchyma (right and basal), the heart, and the functional parenchyma (left and apical) or the heart and mostly bronchi (mediastinal), were pooled together and compared (Fig. 3C). The results from this analysis indicate that for the early phase (Fig. 3C, Early), the left + apical (L + A) and lateral (Lt) parts of the lung were the most sensitive to radiation-induced pulmonary dysfunction and differed from the baseline significantly (P ≤ 0.021) whereas the other regions did not. This changed in the intermediate phase (Fig. 3C, Intermediate) where except for the mediastinal (M) region, no significant differences were observed between the other regions (P = 0.7) although all were significantly elevated above the baseline (P ≤ 0.021). Interestingly, the differences observed in the early phase reappeared during the late phase (Fig. 3C, Late). Thus, regional differences were detected in all postirradiation periods and the left and apical pulmonary regions showed consistently a high sensitivity in terms of functional damage.

Consequentiality between the phases. To examine whether a respiratory deficiency in an earlier period has a consequence in a later period, correlations between individual BRs of irradiated animals were analyzed in Fig. 4. The early BR elevation almost always (62/65 = 95%) led to a dysfunction in the intermediate phase. However, the intermediate BR increase frequently developed without the antecedent early increase (42/100 = 42% Fig. 4A). Still, the intermediate dysfunction was significantly more likely in the rats that suffered the early dysfunction than in the rats that did not (OR, 28.5; 90% CI, 10.2-79.80). Also the late dysfunction was more probable if the early BR increase occurred (OR, 5.9; 90% CI, 3.0-11.7; Fig. 4B) and even more likely if the intermediate BR increase occurred (OR, 7.4; 90% CI, 3.3-16.9; Fig. 4C). Those results suggest strong consequentiality between the phases. Nevertheless, still a substantial number of new cases of morbidity (not preceded by an earlier morbidity) kept appearing throughout the follow-up.

Six rats suffered a BR increase in the late phase without the antecedent intermediate increase (Fig. 4C). Five of them also lacked the early increase. Interestingly, all these six rats had the heart encompassed in the irradiation field. The OR of developing the late BR elevation in rats with irradiated heart versus rats without irradiated heart was 2.1 (90% CI, 1.1-3.9). The same OR limited to the subgroup of animals showing the intermediate BR increase was 2.4 (90% CI, 1.1-5.3), implicating heart irradiation as an independent risk factor in development of the late respiratory dysfunction.

### Table 1. Comparison between the functional and histologic damage

<table>
<thead>
<tr>
<th>Phase</th>
<th>Region</th>
<th>BR 18-22 Gy (mbpm ± SE)</th>
<th>Histologic score 18-22 Gy (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Apical</td>
<td>196.0 ± 6.1*</td>
<td>2.0 (2-4)</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>194.3 ± 4.6*</td>
<td>2.5 (0-4)</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>191.3 ± 5.2*</td>
<td>2.0 (1-3)</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>177.8 ± 3.4</td>
<td>3.0 (1-4)</td>
</tr>
<tr>
<td></td>
<td>Mediastinal</td>
<td>177.5 ± 3.6</td>
<td>2.0 (2-3)</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>175.8 ± 3.5</td>
<td>2.0 (1-3)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>163.6 ± 3.1</td>
<td>0 (0-0)†</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Left</td>
<td>211.7 ± 7.1*</td>
<td>4.0 (3-4)</td>
</tr>
<tr>
<td></td>
<td>Apical</td>
<td>204.5 ± 6.5*</td>
<td>3.0 (1-4)</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>200.7 ± 5.6*</td>
<td>3.0 (1-4)</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>200.6 ± 5.0*</td>
<td>3.0 (2-3.5)</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>199.1 ± 3.1*</td>
<td>3.5 (3-4)</td>
</tr>
<tr>
<td></td>
<td>Mediastinal</td>
<td>186.2 ± 4.6*</td>
<td>2.5 (2-4)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>163.6 ± 2.7</td>
<td>0 (0-0)†</td>
</tr>
<tr>
<td>Late</td>
<td>Apical</td>
<td>211.8 ± 8.6*</td>
<td>3.0 (2-4)</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>204.9 ± 8.7</td>
<td>3.0 (2-4)</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>191.1 ± 5.7*</td>
<td>3.0 (2-4)</td>
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<td>Basal</td>
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<td>Mediastinal</td>
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<td>Right</td>
<td>172.6 ± 3.4</td>
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<tr>
<td></td>
<td>Control</td>
<td>160.8 ± 5.2</td>
<td>0 (0-0)†</td>
</tr>
</tbody>
</table>

NOTE: BR 18-22 Gy, breathing rate of animals from 18 to 22 Gy dose groups pooled together; mbpm = mean breaths per minute; Histologic score 18-22 Gy, structural damage in irradiated lungs of animals from 18 to 22 Gy dose groups evaluated on a scale of 0 to 4. Kruskal-Wallis test or Mann-Whitney U test with the Bonferroni correction was used to derive the P values.

*Different from the control group (P ≤ 0.05).

†Lower than the other regions (P ≤ 0.05).
Figure 3. Mean BR after 50% lung volume irradiation. A, dose-dependent BR increases in time—an example of the 50% lateral irradiation cohort: 16 Gy (○), 18 Gy (□), 20 Gy (△), and 22 Gy (○). Points, mean BR in breaths per minute (bpm) within a dose group of 9 (week 0-8), 7 (week 10-26), or 4 to 5 animals (week 28-38); bars, SE. Baseline BR (●) fluctuates around 163.7 ± 0.7 bpm. B, BR increases seen in 22 Gy dose groups of right (●), left (□), apical (△), basal (△), mediastinal (●), and lateral (○) irradiation cohorts. Points, mean BR in breaths per minute (bpm) within a group; bars, SE. Three phases of BR dynamics can be distinguished and are marked between 6 and 12 weeks (early phase: the first BR elevation), 16 and 28 weeks (intermediate phase: generalized BR elevation), and 34 and 38 weeks (late phase: suboptimal recovery). Baseline BR (●) fluctuates around 163.7 ± 0.7 bpm throughout the follow-up. C, regional differences in the respiratory dysfunction. Mean BRs in 18 to 22 Gy dose groups when cohorts with similar structures encompassed in their irradiation portals were pooled together: a half of the alveolar parenchyma in the right and basal cohorts (R+B); a half of the alveolar parenchyma plus the heart in the left and apical cohorts (L+A); the heart plus minimum of the alveolar parenchyma in the mediastinal cohort (M); and a majority of the alveolar parenchyma in the lateral cohort (Lt). The BRs from the early (6-12 weeks), intermediate (16-28 weeks), and late (34-38 weeks) phases are shown separately. Bars, SE. Dashed lines, cutoffs for elevated BR values (= baseline BR + 2SD): 182.5, 179.8, and 188.1 bpm in the early, intermediate, and late periods, respectively. Note the regional differences in the respiratory dysfunction following fixed range of doses delivered to the identical percentage (50%) of the total lung volume. *, difference from the baseline (P = 0.05); □, spans over cohorts that mutually differ (P < 0.05); †, cohort that is lower than the remaining cohorts (P < 0.05). P values adjusted by the Bonferroni correction.
A trend was observed that heart irradiation increased the likelihood of development of the early dysfunction (the OR for the early elevation in rats with versus without irradiated heart, 1.3; 90% CI, 0.8-2.3) whereas no influence of heart irradiation could be discerned in the intermediate phase (OR, 0.7; 90% CI, 0.4-1.3). Thus, atop the clear predisposing link between the consecutive phases of morbidity, heart irradiation conferred an extra risk of developing the late, and likely, also the early respiratory deficiencies.

Discussion

New external irradiation techniques emerging in clinical radiotherapy allow high precision of dose delivery to a tumor. Although experimental approaches to such therapies reveal that it is not trivial how and where the dose is deposited around the tumor, knowledge on optimal dose distributions in critical normal tissues is still largely lacking. In the current study, we identify risk factors for pulmonary injury after local irradiation of six variably placed 50% subvolumes in the rat lung. Our findings should serve as an impulse for revision of currently adopted clinical practice estimating risk of complications after a treatment based on dosimetric variables disregarding the spatial information on dose deposition within an organ.

Our data reveal three phases of impairment in respiratory function paralleled by dose-dependent structural abnormalities in the irradiated lung parenchyma. Acute exudative inflammation indicative of pneumonitis in the early phase is followed by chronic productive inflammation ending in fibrosis during the intermediate and late phases. Whereas a strong consequentiality link was noted between the three consecutive phases, still a substantial number of animals that got over the early pneumonitis phase without obvious symptoms became symptomatic during the onset of fibrosis. This is all in agreement with broadly reported manifestation of post-irradiation pulmonary injury in both experimental (28–30) and clinical (3, 4, 8) settings and supports the notion that the level of damage required for the manifestation of fibrosis is lower than that required for the manifestation of pneumonitis (4, 8, 10, 31).

The severity of histologic damage did not vary significantly among the six regions irradiated in this study. This is in agreement with previous findings in mice (19) and pigs (32). Despite the equality in the degree of histologic damage, the six irradiated regions differed in the degree of their functional response. This indicates that regional hypersensitivity to radiation on the level of lung function is not due to hyperradiosensitivity of the tissue or cells in these regions in terms of inflammatory response or extracellular matrix formation. Rather, anatomic or physiologic reasons may underlay these differences in regional response. In our experiments in rats (27, this study), the irradiation of the apical and left regions had the most severe impact on respiratory function, particularly in the early and late phases of the follow-up. This contradicts the results of experiments carried out on mice where irradiation of subvolumes in basal lung had more severe functional consequences than irradiation of the same subvolumes in the apex (19, 20). In the latter studies (19–21), the varying proportion of alveoli, critical for gas exchange function, relative to air conducting bronchi, was indicated as a reason for lower sensitivity of the apex (containing large airways branching from pulmonary hila) and higher sensitivity of the base (containing mostly alveolar parenchyma). This is supported by our data as we find that the region containing the largest proportion of parenchymal tissue (the lateral region) belonged to the more sensitive during all phases. However, it cannot explain why the
lungs apex appeared hypersensitive in rats but resistant in mice although the airway structures of both species are similar. A technical cause for the differences could be dose delivery precision, which likely is higher in our studies due to the larger size of rats than of mice. Biologically, the position of the heart in the irradiation field could play a role. Although it was claimed that cardiac complications were not responsible for the observed differences between the apex and base in mouse lungs, it must be stated that these functional measurements were done 22 weeks after irradiation, which exactly matches the period (the intermediate phase) during which we also describe minimal impact of heart irradiation on the BR.

Also in rats it has been reported that the lower lung was more radiosensitive than the upper lung. The end point in these studies, however, was DNA damage as measured by the micro-nucleus assay in fibroblasts isolated from lungs 18 hours after irradiation. These data were suggested to provide an explanation for the functional response in mice. However, as we now show, the lower lung in rats is not more radiosensitive than the upper lung, and thus no correlation between the DNA damage (of individual cells in this region) and functional responses seems to exist.

In general, local heart irradiation is known to affect respiratory function (36) whereas lung irradiation induces changes in pulmonary vascular bed that cause pulmonary hypertension and lead to a congestive right cardiac failure in humans (4, 37), dogs (38), mice (39), and rats (40). The described hypersensitivity of the apical and left regions correlates closely to the CT-verified inclusion of the heart in these fields (99.5% and 74.5%, respectively). Thus, it is possible that differences in the anatomy, positioning, and accuracy of dose delivery could explain the discrepancies between rats and mice mentioned above.

The sensitizing influence of heart irradiation could well explain the differences in between the apical and left cohorts, both representing a combined damage to the heart and lung parenchyma, and the right and basal cohorts with almost an equal amount of “functional” alveolar parenchyma but no heart in the irradiation field. However, the heart irradiation could not be the sole factor because the mediastinal exposure, encompassing 97.3% of the heart, produced the least sensitive response in terms of BR changes.

It must be realized, however, that the mediastinal region contains mostly large bronchi and vessels around the pulmonary hilus and only a small proportion of alveolar parenchyma. The strong impact of the proportion of the alveolar tissue irradiated on the degree of response is shown by the large effect of the lateral irradiation where no heart is in the field but more than 50% of the critical alveolar tissue is being irradiated (no large airways appear in this field). So, both the proportion of heart and proportion of alveolar tissue in the irradiation field need to be jointly taken into account when predicting the risk of functional damage after lung irradiation. These effects of combined injuries to both organs point towards a synergy between radiation damages in lung and heart. Lung possesses substantial reserve capacity. Redistribution of perfusion to, or a compensatory expansion of, previously underused alveoli may maintain an adequate gas exchange after destruction of irradiated regions (41). Also in the case of failing cardiac function, compensatory abilities have been shown either on the level of the heart itself or on the level of regulatory mechanisms of the entire cardiovascular system (42, 43). Thus, it is plausible that limited damage to each of the two organs separately would remain subclinical, but its combination will exceed compensatory abilities and translate into clinically manifest symptoms.

Interestingly, the impact of heart irradiation was most dramatic during the late phase (OR, 2.1), whereas only a trend (OR, 1.3) was observed during the early phase. This is likely caused by the inclusion of the data on mediastinal region that was resistant due to minimal alveolar tissue involvement. During the intermediate phase, the influence of the heart irradiation disappeared (OR < 1). An explanation of this phenomenon may be derived from observations of early decline in cardiac function after heart irradiation followed by a period of recovery (likely coinciding with the intermediate phase) that precedes the onset of late symptoms reported for rats (43–45), dogs (38), and even humans (46, 47). If true, this would change the traditional view of heart as a late-acting organ and may bear relevance for clinical practice where the combination of subclinical injuries to lung and heart may give rise to unexpected toxicity, both early and late after the radiation treatment. A separate study is under way to more specifically test this hypothesis.

In summary, we report regional differences appearing during the three phases of radiation-induced impairment of respiratory function. These differences depend on the irradiated volume of critical gas exchange pulmonary structures and on the involvement of heart in the irradiation field. This urges more attention to possible combined effects of lung and heart irradiation in clinical practice. Our data also imply that simple dosimetric variables, like mean lung dose, which do not take into account spatial distribution of radiation dose within an organ and yet are endorsed for risk estimation in clinics (12), cannot be safely used for prediction of respiratory complications following modern radiation techniques like intensity modified radiation therapy or proton irradiations.

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