Radioprotection of Lung Tissue by Soy Isoflavones

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Introduction: Radiation-induced pneumonitis and fibrosis have restricted radiotherapy for lung cancer. In a preclinical lung tumor model, soy isoflavones showed the potential to enhance radiation damage in tumor nodules and simultaneously protect normal lung from radiation injury. We have further dissected the role of soy isoflavones in the radioprotection of lung tissue.

Methods: Naive Balb/c mice were treated with oral soy isoflavones for 3 days before and up to 4 months after radiation. Radiation was administered to the left lung at 12 Gy. Mice were monitored for toxicity and breathing rates at 2, 3, and 4 months after radiation. Lung tissues were processed for histology for in situ evaluation of response.

Results: Radiation caused damage to normal hair follicles, leading to hair loss in the irradiated left thoracic area. Supplementation with soy isoflavones protected mice against radiation-induced skin injury and hair loss. Lung irradiation also caused an increase in mouse breathing rate that was more pronounced by 4 months after radiation, probably because of the late effects of radiation-induced injury to normal lung tissue. However, this effect was mitigated by soy isoflavones. Histological examination of irradiated lungs revealed a chronic inflammatory infiltration involving alveoli and bronchioles and a progressive increase in fibrosis. These adverse effects of radiation were alleviated by soy isoflavones.

Conclusion: Soy isoflavones given pre- and postradiation protected the lungs against adverse effects of radiation including skin injury, hair loss, increased breathing rates, inflammation, pneumonitis and fibrosis, providing evidence for a radioprotective effect of soy.

Key Words: Soy, Radiation, Lung, Protection.

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Radiotherapy for non–small-cell lung cancer (NSCLC) is limited by radiation toxicity to normal lung tissue, which results from an inflammatory process caused by damage to capillary endothelial cells and epithelial lung cells causing pneumonitis and fibrosis.[1–4]

We are exploring the potential for biological/nutritional intervention using soy isoflavones to boost the success of radiotherapy and decrease its toxicity in NSCLC. This complementary approach could benefit patients with compromised lung functions including chronic obstructive pulmonary disease in smokers and patients with larger or nonperipheral tumors, such as unresectable stage III locally advanced NSCLC, in which radiotherapy is limited by anticipated toxicity.[1] Soy isoflavones are nontoxic dietary plant estrogens extracted from soy beans and anticancer agents, as demonstrated in controlled clinical trials.[5] They can also act as antioxidants in normal tissues and protect them from treatment-induced toxicity.[6] This protective effect was observed in our clinical trial for prostate cancer patients, showing that soy isoflavone pills, taken in conjunction with radiotherapy, reduced radiation toxicity, resulting in improved urinary, gastrointestinal, and sexual functions.[6]

The rationale for selecting soy isoflavones to combine with radiotherapy for NSCLC is based on our work demonstrating that these compounds have a differential effect on tumor versus normal tissue. In contrast to normal cells, critical survival pathways are constitutively activated in cancer cells, including DNA-repair molecules, the nuclear factor-κB, and hypoxia-inducible factor-1α transcription factors, which are responsible for promoting malignant behavior by transcription of proteins involved in tumor progression. These survival pathways are further up-regulated in response to radiation and thereby, are implicated in radioresistance of cancer. We have consistently shown that soy isoflavones inhibited radiation-induced up-regulation of these survival pathways resulting in greater cancer destruction both in vitro and in vivo, using orthotopic models of lung carcinoma, renal cell carcinoma, and prostate cancer.[7–14] In particular, in lung cancer studies, we previously reported that soy isoflavones enhanced radiation-induced cell killing of human NSCLC cells in vitro by increasing DNA damage and inhibiting DNA repair in addition to inhibiting nuclear factor-κB and hypoxia-inducible factor-1α.[12] In contrast, normal cells do not express such activated malignant survival pathways and thus, are not affected directly by soy isoflavones. In normal tissues, radiation causes tissue damage, resulting in inflammatory processes leading to pneumonitis and fibrosis. Initial studies suggested that soy inhibited the progression of radiation-induced inflammatory

events in normal lung tissue. In an orthotopic murine model of lung cancer, soy isoflavones increased radiation-induced destruction of lung tumor nodules, but also mitigated the vascular damage, inflammation, and fibrosis caused by radiation injury to lung tissue.[7] These studies suggested that soy isoflavones have the dual potential to enhance radiation damage in lung tumors and simultaneously protect normal lung from radiation injury.

To evaluate further the role of soy isoflavones in moderating adverse effects of radiation on lung tissue, we have now investigated the effect of soy isoflavones on the damage caused by a high-radiation dose in normal lung in naive mice not bearing tumors. These conditions were selected to detect significant tissue damage by high-radiation doses, which have been shown to result in greater toxicity in clinical studies.[15] We report that supplementation with soy isoflavones pre- and postradiation clearly attenuated skin injury and hair loss caused by radiation, a cogent evidence for the radioprotective effect of soy isoflavones on normal tissues. Furthermore, soy isoflavones protected mice from radiation-increased breathing rate. Histological observation of lung tissues confirmed that soy isoflavones protected normal lung structures against radiation-induced inflammation, damage, and fibrosis.

MATERIALS AND METHODS

Mice

Female Balb/c mice that were 5 to 6 weeks old (Harlan, Indianapolis, IN) were housed and handled in animal facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The animal protocol was approved by Wayne State University Institutional Animal Care and Use Committee.

Soy Isoflavones

The soy isoflavones mixture G-4660 used is a pure extract of 98.16% isoflavones from soybeans consisting of 83.3% genistein, 14.6% daidzein, and 0.26% glycitein (manufactured by Organic Technologies and obtained from National Institutes of Health, Bethesda, MD). The soy isoflavones mixture was dissolved in 0.1 mol/liter Na_2CO_3 and mixed with sesame seed oil at a 2:1 ratio just before treatment to facilitate gavage and avoid irritation of the esophagus by Na_2CO_3.[7] Mice were orally treated with 5 or 1 mg/day of soy isoflavones (250 or 50 mg/kg body weight/day) by gavage. Control mice received the vehicle alone.

Lung Irradiation

Radiation was delivered to the left lobe of the lungs. Three anesthetized mice, in jigs, were positioned under a 6.4-mm lead shield with three cutouts in an aluminum frame mounted on the radiograph machine to permit selective irradiation of the left lung in three mice at a time, as previously described.[7] The radiation dose to the lung and the scattered dose to areas of the mouse outside of the radiation field were carefully monitored. To minimize backscattering of radiation, the bottom of the aluminum frame that holds the jigs was hollowed out and the backplate of the jig was thinned to 1.6-mm

thickness. Under these conditions and the lead shielding, the radiograph dose to the shielded regions was reduced to 1% of the dose to the irradiated field. The dose rate was 101.0 cGy/minute and half value layer was 2-mm Cu. Photon irradiation was performed at a dose of 12 Gy with a Siemens Stabilipan X-ray set (Siemens Medical Systems, Inc., Erlangen, Germany) operated at 250 kV, 15 mA with 1-mm copper filtration at a distance of 47.5 cm from the target.

Experimental Protocol

Mice were pretreated with oral soy isoflavones each day for 3 days at a dose of 5 mg/day (equivalent to 250 mg/kg). Then, the left lung was selectively irradiated with 12 Gy. Soy treatment was continued on a daily basis for 5 more days at 5 mg/day. Then mice were treated with a lower dose of 1 mg/day (equivalent to 50 mg/kg), given daily 5 days a week for up to 18 weeks. The rationale for giving a higher dose of soy isoflavones for pretreatment and just after radiation was optimization of the effect of soy, based on previous studies.[8,10]

Hair Loss Monitoring of Mice Treated with Soy Isoflavones and Lung Irradiation

Mice were treated with soy isoflavones and left lung irradiation as described above (see Experimental Protocol section). After radiation, mice were treated with soy given daily, 5 days a week, for 5 more weeks. Four experimental treatment groups consisting of 13 mice per group included control mice treated with vehicle alone, mice treated with soy alone, mice treated with radiation alone and mice treated with soy and radiation. Mice were monitored three times a week for signs of radiation-induced injury. The area showing hair loss was measured in two dimensions. Hair loss in the radiated field was scored at 100% for bare skin, to 75%, 50%, and 25% based on the density of hair and the size of area showing hair loss.

Breathing Rate Measurements of Mice Treated with Soy Isoflavones and Lung Irradiation

Mice were treated with soy isoflavones and left lung irradiation as described above (see Experimental Protocol section). After radiation, mice were treated with soy given daily, 5 days a week, for up to 18 weeks. Four experimental treatment groups consisting of eight mice per group included control mice treated with vehicle alone, mice treated with soy alone, mice treated with radiation alone, and mice treated with soy and radiation. Mice were monitored for up to 4 to 5 months and their breathing rate was measured using a mouse CollarClip Sensor according to manufacturer's instructions (STARR Life Sciences, Corp., Oakmont, PA).[16–18] The breathing rate of eight mice per treatment group was measured at late time points of about 2, 3, and 4 months (days 59, 86, and 130) after radiation. Data were collected on conscious mice during 10 minutes for each mouse. Breathing rates were measured when mice were relatively calm and no error code was shown on the instrument.[16] These rates were computed to estimate the average breathing rate of each mouse.

Lung Tissue Preparation for Histological Examination

Mice were treated with soy isoflavones and left lung irradiation as described above (see Experimental Protocol section). After radiation, mice were treated with soy given daily, 5 days a week, for up to 18 weeks. At different time points, mice were killed and lungs were perfused with 10% buffered formalin for fixation in situ. The lungs were resected and the irradiated left lung and the nonirradiated right lung were processed separately for paraffin embedding and sectioning.[7] Sections were stained with hematoxylin and eosin. The extent of fibrosis was evaluated by staining lung sections with Masson's Trichrome (NovaUltra Kit; IHCWORLD, Woodstock, MD).[7,19] The extent of lung damage, inflammatory infiltration, pneumonitis, and fibrosis were quantified using a scoring system on a scale from weak (±), moderate (+), strong, (++) to heavy (+++).

Statistical Analysis

For histological data analysis, differences in the surface area of hair loss and breathing rates among the various treatments groups were analyzed by two-tailed unpaired Student's t test.

RESULTS

Cogent Evidence Demonstrating Radioprotection by Soy Isoflavones: Decrease in Radiation-Induced Hair Loss

To determine whether soy isoflavones could protect normal lung tissues from radiation-induced damage, mice were treated with radiation alone or combined with soy isoflavones for up to 7 weeks, as detailed in Materials and Methods section. Mice were monitored daily for signs of toxicity. We observed hair loss in the left upper thoracic side, which had been irradiated. Hair loss was scored as 100%, 75%, 50%, and 25% in the irradiated area based on the size of the injured area and the hair density (Fig. 1). For example, in mice treated with radiation alone, a mouse with bared skin was scored as having 100% hair loss and most of the mice showed 50% to 75% hair loss (Fig. 1A). In contrast, a large number of mice in the experimental group treated with soy isoflavones before and after radiation showed 0% to 25% hair loss (Fig. 1B). The proportion of mice with significant hair loss in the radiation group was 13 of 13 mice, with 92% of the mice showing 50% or greater hair loss (Fig. 2A). However, in the experimental group treated with soy isoflavones and radiation, only six of 13 mice had measurable hair loss, mostly in the range of 25% (Fig. 2A). Kinetics experiments showed that hair loss appeared as early as 3 weeks after radiation and all mice showed hair loss by 5 weeks after radiation (Fig. 2B). The surface area with hair loss progressively increased with time in all mice treated with radiation only (Fig. 2B). However, the kinetics of hair loss were much slower in mice treated with radiation combined with soy isoflavones beginning at 4 to 6 weeks after initiation of soy treatment (Fig. 2C). The surface area of hair loss was also significantly smaller by 5 to 6 weeks compared with that of mice treated with radiation alone ($p < 0.01$; Fig. 2C).

Soy Isoflavones Mitigated the Increased Breathing Rate Induced by Radiation

Lung tissue injury induced by radiotherapy leads to an inflammatory process that results in pneumonitis and fibrosis.[20,21] The presence of fibrosis in lung tissues leads to
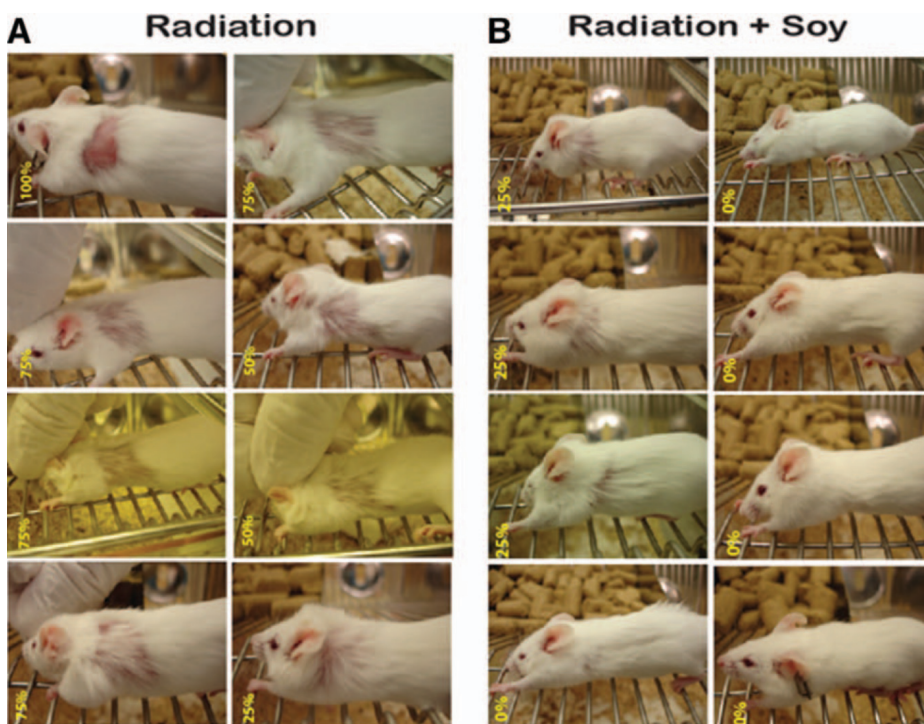

FIGURE 1. Hair loss in irradiated thoracic area of mice treated with radiation or radiation combined with soy isoflavones. *A,* Hair loss induced by radiation. Mice received 12 Gy radiation to the left lung. *B,* Protection against radiation-induced hair loss by soy isoflavones. Mice were treated with soy isoflavones and 12 Gy radiation administered to the left lung. Soy was given 3 days before radiation and continued 5 days a week after radiation. At about 7 weeks after initiation of soy treatment, hair loss was scored from 0% to 100% as shown in yellow numbers on the bottom left of each mouse picture. Eight representative mice per treatment group are shown.
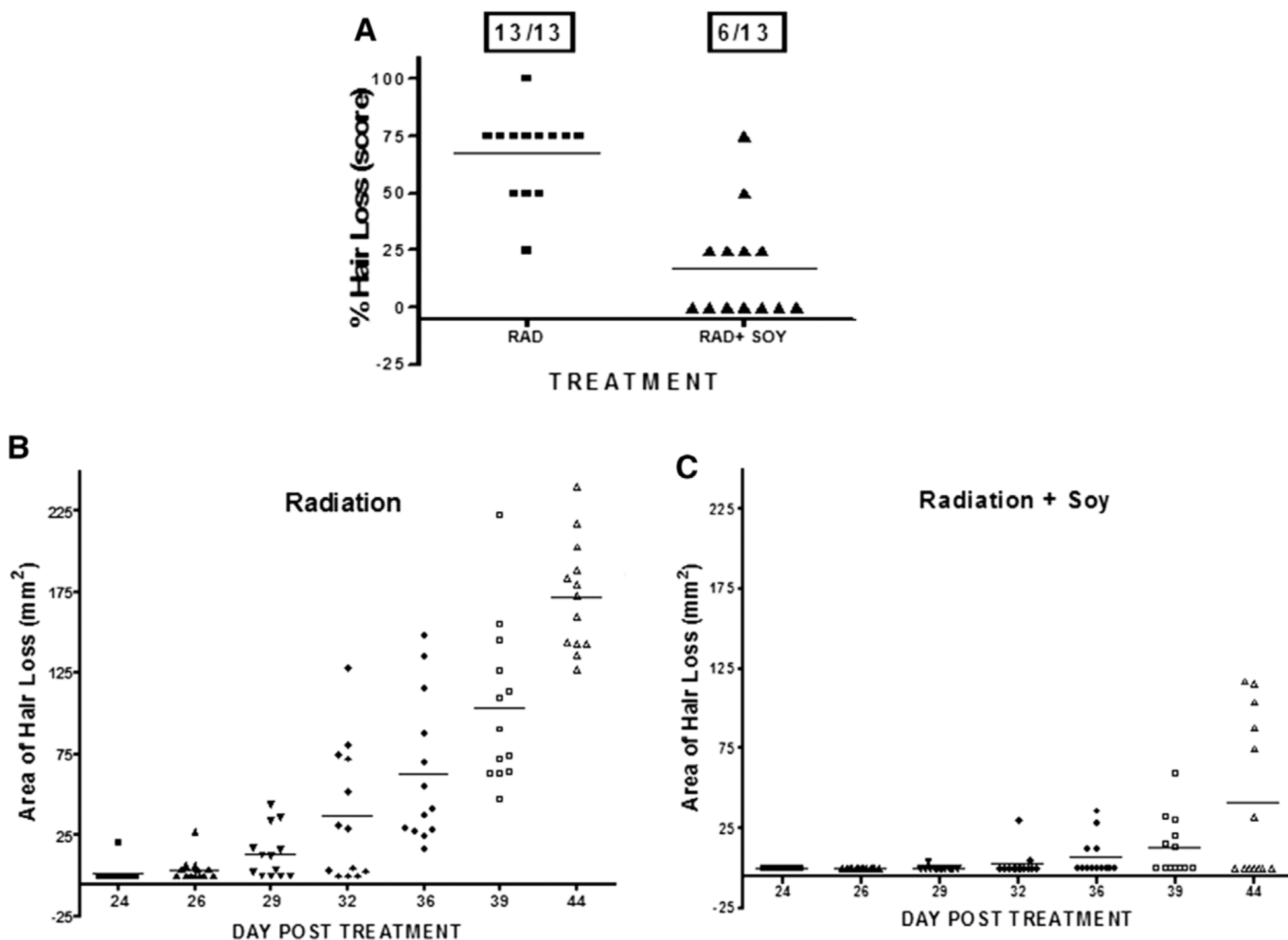
FIGURE 2. Kinetics of hair loss in irradiated thoracic area of mice treated with radiation combined with soy isoflavones. Mice were treated either with 12 Gy radiation administered to the left lung alone or combined with soy isoflavones treatment. Soy was given 3 days before radiation and continued 5 days a week after radiation. *A*, Hair loss scoring in mice treated with radiation or radiation and soy isoflavones. At about 7 weeks after initiation of soy treatment, hair loss was scored from 0% to 100% based on the density of hair and the size of area showing hair loss. The proportion of mice with hair loss is based on 13 mice per treatment group (inserted in text box). *B* and *C*, Kinetics of hair loss after radiation (*B*) or radiation combined with soy isoflavones treatment (*C*). Mice were monitored for hair loss three times a week after initiation of treatment. The area showing hair loss was measured in two dimensions and the surface area was plotted. Each dot in *A*, *B*, and *C* represent one mouse.

difficulties in breathing, which cause increased breathing rate and these late effects of radiation toxicity occur in patients several months after radiation.[3,22] Increase in breathing rates was also documented in mice or rats treated with lung irradiation.[2,23–25] To evaluate the effect of soy isoflavones on breathing rates altered by radiation, we have measured the breathing rate of mice treated with lung irradiation, soy isoflavones, and both combined. An increase in the breathing rate of mice receiving only lung irradiation was noticed by days 59 and 86 (Fig. 3). The increased breathing rates were significantly more pronounced by 4 months (day 130) after radiation with a mean of 209 ± 19 breaths/minute compared with 170 ± 8 breaths/minute in control mice ($p < 0.001$), probably because of the late effects of radiation injury to lung tissue (Fig. 3). However, this effect was mitigated by supplementation with soy isoflavones pre- and

postradiation, causing a breathing rate in the lower range of 168 ± 5 breaths/minute ($p < 0.001$; compared with radiation) that was comparable with that of untreated control mice (Fig. 3).

Histological Observations in Lungs Treated with Soy Isoflavones and Radiation

To assess in situ the effect of soy isoflavones and left lung irradiation on lung tissue structures, both the left lung and the right lung were separately resected at about 2, 3, and 4 months after treatment with either therapy alone or both combined and processed for histology studies. At all time points, lungs from control mice showed normal blood vessels with integral basement membrane, endothelial lining with endothelial cells surrounded by normal alveoli (Fig. 4*A1*). Normal structures of bronchioles lined by ciliated columnar epithelium and
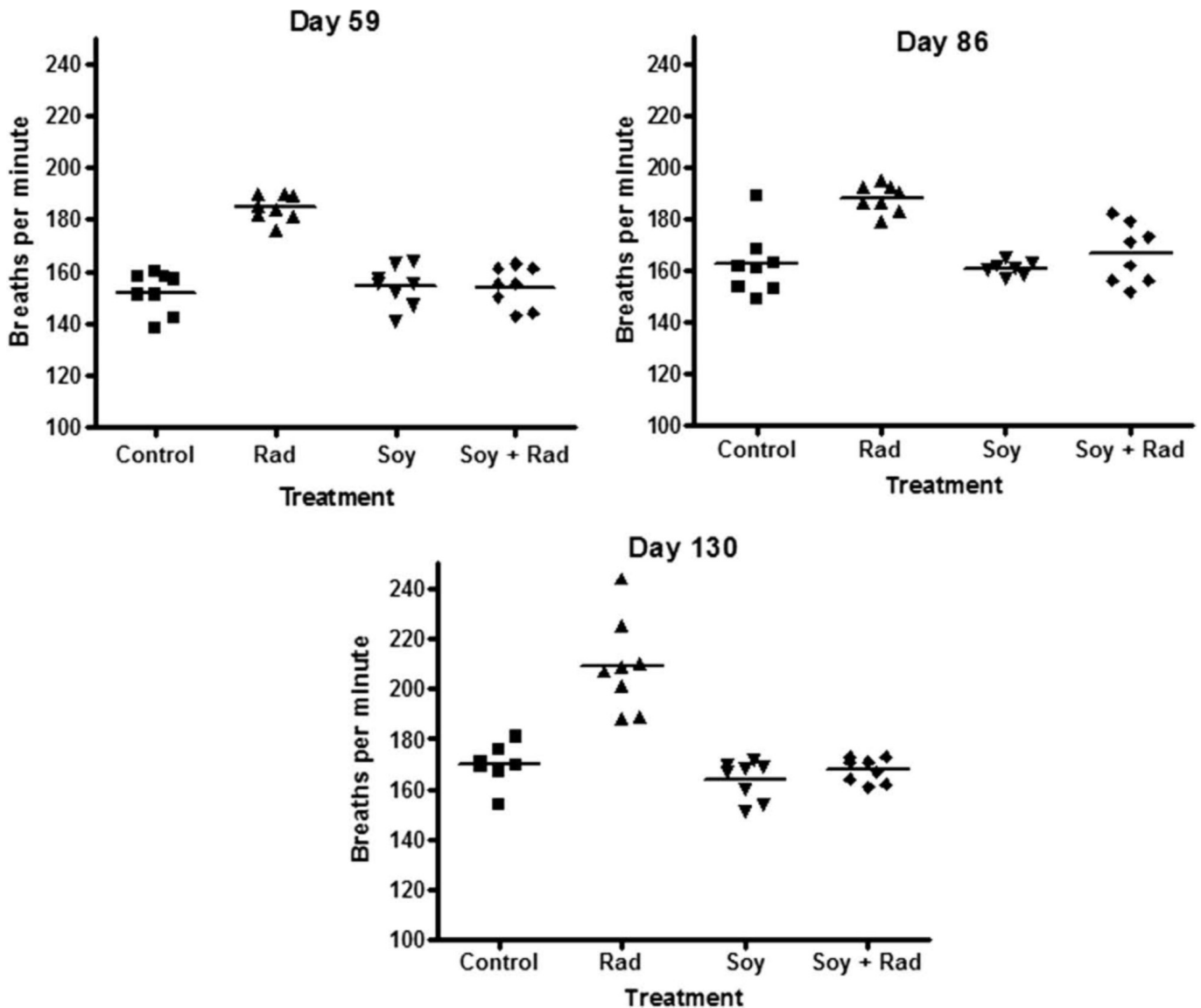
FIGURE 3. Breathing rate of mice treated with soy and radiation. Mice were treated either with soy or 12 Gy radiation (Rad) administered to the left lung alone or soy isoflavones combined with radiation (Soy + Rad). Soy was continued 5 days a week for up to 18 weeks. The breathing rate of eight mice per treatment group was measured on day 59, 86, and 130 after initiation of soy treatment. Each dot represents the average breathing rate value obtained from one mouse (*n* = 8 per group).

surrounded by normal thin alveolar septa were seen (Fig. 4*A2*, *A3*; Table 1). After treatment with soy isoflavones at 2, 3, and 4 months, the structures of blood vessels (Fig. 4*B1, B2*), bronchioles and the thickness of alveolar were not altered and looked normal compared with that of untreated lungs (Fig. 4*B1–B3*; Table 1). In contrast, radiation to the left lung caused significant tissue damage, which was clearly observed on day 64 and further increased at the later time points of day 87 and 134 (Table 1). The alveolar septa and vessel walls showed a chronic inflammatory infiltrate indicative of pneumonitis, causing a marked reduction in alveolar spaces compared with control or soy-treated lungs (Fig. 4*C1–C3*; Table 1). Clusters of inflammatory cells were observed in the vicinity of blood vessels and infiltrating into the alveoli (Fig. 4*C1, C3* and *C1A,*

C3A), disrupting the integrity of the vessel wall (Fig. 4*C2*). Focal areas of damaged alveolar tissues were replaced by a heavy infiltration of inflammatory infiltrates consisting mostly of histiocytes and lymphocytes (Fig. 4*C2* and *C2A*; Table 1). Focal hemorrhages were seen, likely the result of vascular damage induced by radiation. These data suggest that radiation caused a long-lasting and strong inflammatory response that became even more prominent by 4 months after radiation. These findings were not observed in nonirradiated right lungs of these mice. Irradiated left lungs from mice treated with soy isoflavones before and after radiation showed mostly normal bronchioles, alveoli, and blood vessels with minimal inflammatory infiltrate and hemorrhage showing milder pneumonitis compared with radiation-treated lungs (Fig. 4*D1–D3*; Table 1).

FIGURE 4. H&E staining of lung tissue sections from mice treated with soy, radiation, and radiation + soy. Mice were treated either with soy or 12 Gy radiation (Rad) administered to the left lung alone or soy isoflavones combined with radiation (Soy + Rad). Soy was continued 5 days a week for up to 19 weeks. Lung tissue sections obtained on days 64, 87, and 134 after radiation were processed for H&E staining. *A1–3*, Control lung from untreated mice showing normal structures of lung tissues including vessels (V), bronchioles (BR), and alveolar septa (AS). *B1–3*, Soy-treated lungs showing also normal structure of lung structures on days 64, 87, and 134 of soy treatment. *C1–3*, Radiation-treated left lung showing thickened alveolar septa and clusters of IF in the vicinity of blood vessels (*C1*, *C3*) and infiltrating into the alveoli (*C2*) as seen in *C.1A*, *C.2A*, and *C.3A* enlarged areas. Focal areas of damaged alveoli tissues were replaced by a heavy infiltration of inflammatory infiltrates consisting mostly of histiocytes and lymphocytes (*C2*). *D1–3*, Radiation + Soy–treated lungs showing less thickening of alveolar septa and less disruptions in lung tissue with reduced inflammatory infiltrates compared with radiation alone. All magnifications ×20. H&E, hematoxylin and eosin; IF, inflammatory infiltrates.
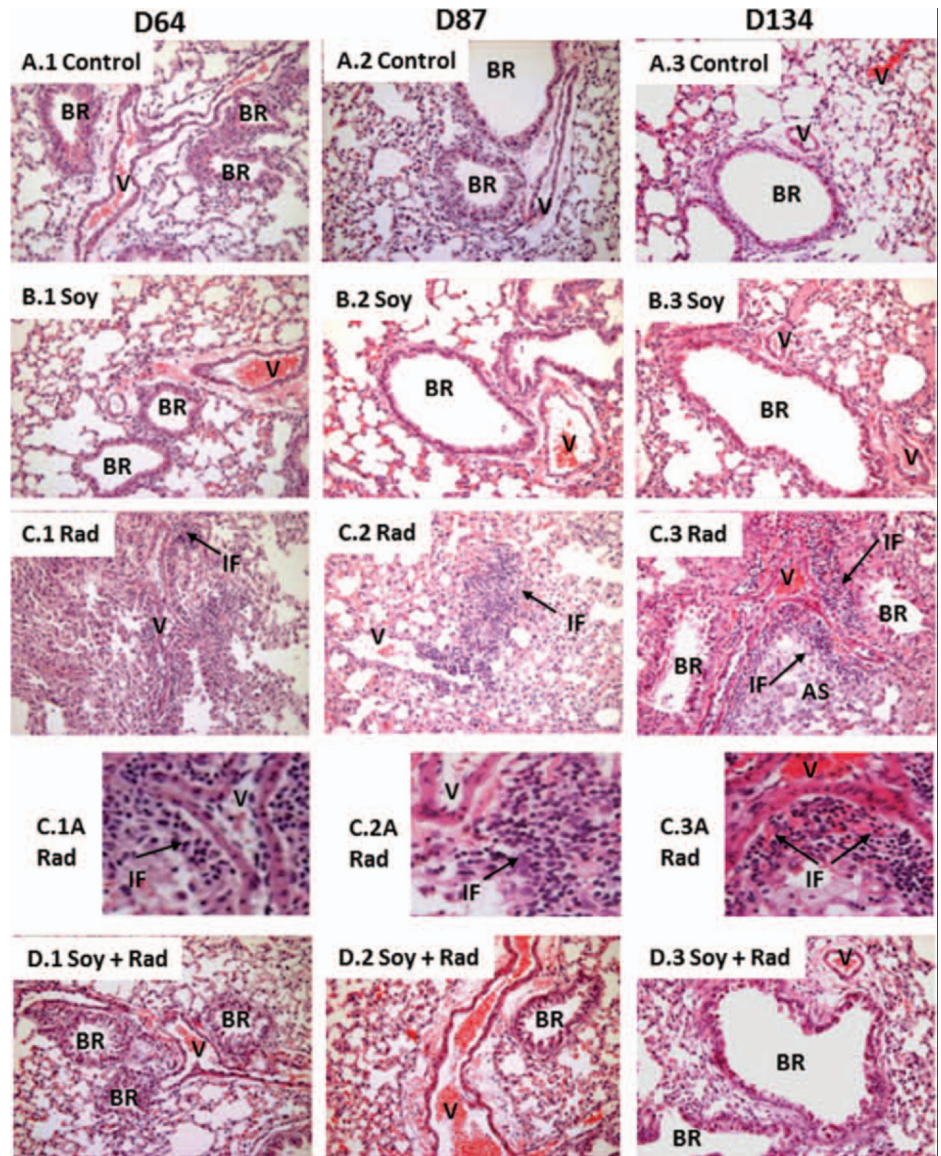
Detection of Fibrosis in Lungs Treated with Soy and Radiation

We have documented above the occurrence of radiation-induced pneumonitis as well as the beneficial effects of soy supplementation. Because pneumonitis is associated with development of fibrosis,[4,20] the effect of soy isoflavones on this late event was also evaluated by staining collagen fibers with the Masson's trichrome stain. The lungs from control and soy-treated mice showed typical light blue staining of collagen fibers around vessels and bronchioles (Fig. 5*A1*, *A2* and

TABLE 1. Summary and Quantification of Histological Observations

	Lung Damage			IF Cells			Pneumonitis			Fibrosis		
	D64	D87	D134	D64	D87	D134	D64	D87D	D134	D64	D87	D134
Control	±	±	+	±	±	+	±	±	+	±	±	+
Soy	±	±	+	±±		+	±	±	+	±	±	+
Radiation	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	++	+++
Rad + Soy	±	±	+	±	±	+	±	±	+	±	±	++

Histological findings from the experience presented in Figures 4 and 5 were quantified. The extent of lung damage (disruption of alveoli, bronchioles, and vessels), IF cells, pneumonitis (thickened septa), and fibrosis were scaled from weak (±), moderate (+), strong (++), to heavy (+++).
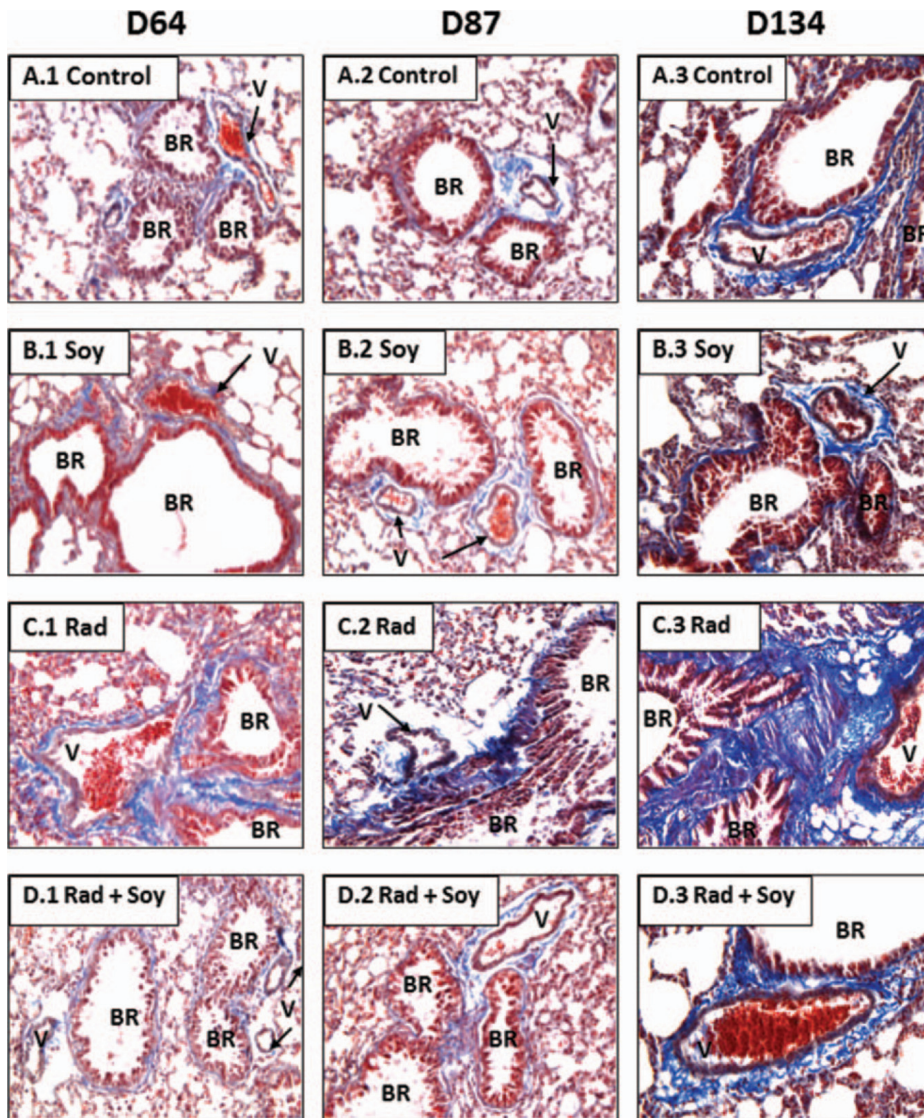
IF, inflammatory infiltration.

FIGURE 5. Soy inhibition of radiation-induced fibrosis in bronchovascular bundles. Lung tissue sections obtained on days 64, 87, and 134 after radiation from the experiment described in Figure 4 were processed for Masson's trichrome staining for detection of fibrosis. *A1–3*, Control lungs from untreated mice showing regular pattern of thin collagen fibers (stained in blue) around vessels (V) and bronchioles (BR) in lung tissue. *B1–3*, Lungs from soy-treated mice showing regular pattern of collagen staining like in control mice. *C1–3*, Radiation (Rad) caused a marked increase in density of collagen fibers around vessels and bronchioles in left lung tissues, which increased with time being extensive by day 134 after radiation. *D1–3*, Lung tissues treated with radiation and soy (Rad +Soy) showed normal bronchovascular collagen fibers similar in density to that observed in control lungs on days 64 and 87. It should be noted that by day 134 of the experiment the lungs were more congested in all treatment groups with a higher density of bronchovascular collagen fibers, however, there was a striking increase in fibrosis staining in radiation-treated lungs compared with control, soy, and Rad + Soy–treated lungs. All magnifications ×20.

B1, *B2*; Table 1) although the staining was more intense at a later time point on day 134 (Fig. 5*A3, B3*; Table 1). Left lung irradiation caused an increase in collagen fibers supporting vessel walls and bronchioles seen on day 64 (Fig. 5*C1*; Table 1) compared with control lungs. The intensity of collagen stain was further increased at later time points on days 87 and 134 (Fig. 5*C2, C3*; Table 1). By day 134, an intense blue stain of bronchovascular bundles was clearly observed (Fig. 5*C3*; Table 1), suggesting progression of fibrosis. Lungs from mice treated with irradiation and soy isoflavones showed, within the bronchovascular bundles, an amount of collagen comparable with that observed in control lungs at the three time points tested (Fig. 5*D1–D3*; Table 1).

DISCUSSION

The process of lung injury caused by radiation is unique for this particular organ because of the clinical consequences of the pathological progression of normal lung tissue to inflamed lung and fibrotic tissue, thereby impeding

normal breathing functions. Radiation pneumonitis is caused by an early inflammatory process triggered by damage to lung parenchyma, epithelial cells, vascular endothelial cells, and stroma, which involves induction of proinflammatory cytokines and chemokines that recruit inflammatory immune cells in the lung tissue.[2,3,26,27] This acute early pneumonitis actually progresses to a chronic inflammation mediated by cyclical phases of cytokines, chemokines, and growth factors released in the tissue microenvironment.[3,28] These complex events culminate in the later stage of lung fibrosis, which result from excessive accumulation of collagen and other extracellular matrix components.[3,4,28] These adverse events of radiotherapy affect patients' breathing and their quality of life.[1,20,22,28]

In the current study, we explored whether supplementation with natural and safe soy isoflavones could improve high-dose radiotherapy for inoperable NSCLC by alleviating radiation injury to normal tissues. To focus our efforts on dissecting the effect of soy isoflavones on radiation injury to

normal lung tissue, we decided to study this complementary approach in naive mice not bearing tumors.

Radiation caused damage to normal hair follicles, leading to hair loss in the irradiated left thoracic area. Supplementation with soy isoflavones pre- and postradiation protected the majority of the mice against radiation-induced skin injury and hair loss or reduced the extent of hair loss. These original observations represent cogent evidence that soy isoflavones protect normal tissues from radiation-induced damage. Radiation-induced skin injury, probably because of damage to stem and progenitor cells, could progress from mild erythema to moist desquamation and ulceration and is still observed mostly in breast cancer patients as well as NSCLC patients because of the proximity between the skin and the target volume.[15,29,30] Our findings on the protective effects of soy suggest that soy could be used to mitigate skin injury induced by radiation in patients. Other antioxidants including nitroxides such as tempol have shown efficacy in reducing alopecia in rodents and in a few patients treated with brain radiotherapy.[31,32] Other studies using mice showed the benefit of using plerixafor, a chemokine receptor type 4 antagonist, as a bone marrow stem cell mobilizer to improve both acute and late skin response to high doses of 25 to 30 Gy radiation.[33]

The radiation-induced acute inflammatory pneumonitis and late pulmonary fibrosis lead to compromised lung function including perfusion and gas exchange, affecting the breathing capacity of the patients.[1,20,22,28] This phenomenon expressed by increased breathing rates in patients was also reported in rats and mice by 2 to 4 months after doses of 10 Gy or more.[2,23,25] In our studies, lung irradiation at 12 Gy caused an increase in breathing rate of mice, which was more pronounced by 4 months after radiation probably because of late effects of radiation-induced injury to normal lung tissue. Supplementation with soy isoflavones before and after radiation blocked the increase in breathing rate. These findings are in agreement with recent studies in rats showing that the genistein isoflavone or the superoxide dismutase catalase mimetic EUK-207 delayed and suppressed radiation-increased breathing rate, confirming the radioprotective effect of genistein, which is the most active component of soy.[24] A natural extract of soy isoflavones containing genistein, daidzein, and glycetein isoflavones was used for the current lung study because it was found to be more potent and safer than purified genistein.[10] The increased breathing rate caused by radiation is associated with pneumonitis.[20] Histological observations of the left lung treated with 12 Gy irradiation showed the occurrence of tissue damage along with an inflammatory response. Irradiated lung tissue showed thickening of alveolar septa, which is indicative of pneumonitis, as well as damage to alveolar structures, bronchioles, and vessel integrity. Focal hemorrhages, probably a result of vascular damage, were seen. A heavy, chronic infiltration of inflammatory cells consisting of histiocytes and lymphocytes was observed involving vessels, bronchioles, and alveoli. These alterations continued to progress up to the later time points of 3 to 4 months after radiation. Concurrently, a progressive increase in fibrosis was also observed in the bronchovascular bundles, suggesting a long-lasting and ongoing radiation-induced tissue damage over time. In contrast, lungs treated with radiation combined with soy showed milder pneumonitis and reduced fibrosis. A decrease in fibrosis caused by genistein or EUK-207 was also observed in irradiated lungs of naive rats that could be associated with antioxidant and anti-inflammatory mechanisms involving inhibition of transforming growth factor-β1 expression, which is a major fibrogenic cytokine.[4,24]

Taken together, these observations confirm that soy isoflavones can modulate the inflammatory response caused by radiation and slow down the progressive tissue damage induced by radiation, which leads to impaired lung function. These findings in naive mice are in agreement with our findings on radioprotection of normal lung tissues in the A549 lung tumor model[7] and demonstrate that soy can protect against radiation-induced injury to normal lung tissue. In our studies, the levels of isoflavones measured in the serum of mice treated with soy isoflavones reflected typical in vivo metabolism with significant levels of daidzein (1.6 μM) and genistein (1.7 μM).[10] These levels are comparable with plasma concentrations of 1 to 4 μM soy isoflavones measured in Asian populations consuming foods rich in soy isoflavones, in contrast to 10 to 30 nM levels found in Western populations.[14]

Several studies on radioprotectors and mitigators to minimize radiation toxicity are ongoing and include radical scavengers and antioxidants, some of which such as amifostine, tempol, and melatonin have been clinically tested.[20,32] However, limitations include their safety and tumor protection from radiation effect. The use of soy isoflavones as radioprotectors is attractive because they were proven to be safe in controlled human clinical trials.[5] Our experimental studies in animal models suggest that the addition of soy to radiotherapy might improve the effect of radiotherapy on the tumor target and reduce the dose-limiting toxicity of radiotherapy to the normal lung. If this proves to be the case, this simple, nontoxic, natural compound would radically improve the effectiveness of this new radiation treatment for inoperable NSCLC.

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