GSN antibody pretreatment aggravates radiation-induced lung injury in mice (postprint)

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Abstract

Radiation-induced lung injury is one of the main dose-limiting factors for thoracic radiation therapy. Gelsolin (GSN) is a ubiquitously expressed, multifunctional regulator of cellular structure and metabolism. In this study, the roles of GSN in radiation-induced lung injury in Balb/c mice were investigated. Plasma GSN levels decreased progressively within 72 hours post-irradiation, and then increased gradually. GSN content in bronchoalveolar lavage (BAL) fluid increased after thoracic irradiation, whereas GSN mRNA levels in lung tissue decreased significantly within 24 hours post-irradiation and then increased again. Mice were intravenously injected with 50 g GSN antibody 0.5 hour before 20 Gy thoracic irradiation. GSN antibody pretreatment increased lung inflammation, protein concentration in BAL fluid, and leukocyte infiltration in irradiated mice. The activities of superoxide dismutase (SOD) in plasma and BAL fluid of irradiated mice injected with GSN antibody were lower than those of control groups, whereas malondialdehyde (MDA) levels increased. These results suggest that GSN antibody pretreatment may aggravate radiation-induced pneumonitis.

Full Text

Preamble

GSN Antibody Pretreatment Aggravates Radiation-Induced Lung Injury in Mice

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Abstract: Radiation-induced lung injury is one of the main dose-limiting factors for thoracic radiation therapy. Gelsolin (GSN) is a widespread, multifunctional regulator of cellular structure and metabolism. In this work, the roles of GSN in radiation-induced lung injury in Balb/c mice were studied. The GSN levels in plasma reduced progressively in the 72 hours after irradiation, and then increased gradually. GSN contents in the bronchoalveolar lavage (BAL) fluid increased after thoracic irradiation, whereas mRNA levels of GSN in the lung tissue decreased significantly within 24 hours after irradiation and then increased again. Mice were intravenously injected with 50 μg GSN antibody 0.5 hour before 20 Gy of thoracic irradiation. GSN antibody pretreatment increased lung inflammation, protein concentration in the BAL fluid, and leukocyte infiltration in the irradiated mice. The activities of superoxide dismutase (SOD) in the plasma and the BAL fluid in irradiated mice injected with GSN antibody were less than that of control groups, whereas the levels of malondialdehyde (MDA) increased. These results suggest that pretreatment of GSN antibody may aggravate radiation-induced pneumonitis.

Keywords: Gelsolin, Thoracic radiation, Lung injury, Acute pulmonary inflammation, Antioxidant ability

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Introduction

Radiation therapy is a common therapeutic modality for thoracic malignancies such as lung, esophagus, and breast cancers. However, large doses of ionizing radiation may induce acute pulmonary inflammation and subsequent pulmonary fibrosis, which causes low quality of life for patients [1, 2]. Radiation pneumonitis may occur within two weeks after the initiation of radiation therapy, thus interrupting treatment. Several months after radiation therapy, excessive fibrotic tissues may replace the inflammatory lung parenchyma and suppress oxygen diffusion capacity. Currently, there are rarely effective clinical methods to treat radiation-induced lung injury. Radiation pneumonitis is commonly treated with glucocorticoids, Amifostine, antibiotics, auxiliary γ-interferon, bronchodilator agents, etc. Chinese medicine can also be applied, but there is no determinate efficacy for late pulmonary fibrosis.

Gelsolin (GSN), a calcium-dependent actin filament severing and capping protein, is an actin-binding protein of particularly high abundance [3–5]. GSN is composed of six structurally homologous domains of 120–130 amino acids, designated (from the N-terminus) as G1–G6 where three different actin binding domains are distributed. GSN can be modeled as two halves (the N-terminal S1–S3 and the C-terminal S4–S6) separated by a 70 amino acid linker sequence. The linker can be cleaved by some proteases [3, 5]. Actin is the fundamental component of cytoskeleton and can be released into circulation by cellular injury.

Circulating actin can interfere with the microcirculation of the lungs and may be directly toxic to pulmonary endothelium. Studies have revealed that plasma GSN participates in the clearance of actin from circulation, and lower levels of GSN are found in many tissue damages, such as sepsis, acute lung injury, end-stage renal disease, and bronchopulmonary dysplasia of prematurity [6–10]. Goetzl et al. [11] and Osborn et al. [12] reported an important function that GSN might inhibit the inflammatory response. They found that the binding of bioactive inflammatory mediators such as platelet-activating factor (PAF) and lysophosphatidic acid (LPA) to GSN attenuated their deleterious effects. PAF and LPA are important mediators of recruiting acute inflammatory cells to sites of injury. The administration of recombinant GSN significantly restrained the inflammatory responses and immune reactions, and thus reduces mortality of animals [7, 9, 13].

Ionizing radiations induce injuries in the epithelial and endothelial cells in the lung and recruit circulating neutrophils into the injury sites. Alveolar macrophages could be activated to release toxic products that exacerbate inflammatory and oxidative injuries [6, 13, 14]. GSN deficiency causes increased pulmonary vascular permeability and exhibits delayed pulmonary neutrophil migration into the lungs upon injury [14–17]. GSN is released by lung epithelial cells into airways, while the mRNA and protein levels of GSN could be increased by interleukin-4 in vitro in lung samples of patients with idiopathic interstitial pneumonia [16, 18]. GSN contributes to the maintenance of vascular barrier function in the lungs and repletion of GSN can partially abrogate the resultant exudative response in the injured lung [13, 15, 17].

To evaluate the roles of GSN in radiation-induced lung injury, GSN antibody was administered to Balb/c mice 0.5 hour before thoracic irradiation. The parameters of lung inflammatory responses and pulmonary vascular permeability were assessed. Our data revealed that pretreatment with GSN antibody aggravates radiation-induced pneumonitis, suggesting a radioprotective role of GSN in radiation-induced lung injury.

II. Materials and Methods

A. Animals

Eight-week-old female Balb/c mice (Silaike Experimental Animal, China) were maintained in a laboratory animal facility with temperature and relative humidity maintained at (23 ± 2)°C and (50 ± 20)%, respectively. All mice were given a standard chow diet and water ad libitum. All experimental procedures were in accordance with the guidelines provided by the Animal Ethical Committee of Soochow University.

Mice were divided into four groups: (1) the control group (control, $n = 15$), (2) the irradiation group (IR, $n = 35$), (3) the irradiation group pretreated with control IgG (IgG, $n = 25$), and (4) the irradiation group pretreated with GSN antibody (anti-GSN, $n = 25$).

B. Irradiation

The mice were anesthetized with 7% chloral hydrate (3.5 ml kg^{-1}) via intraperitoneal injection. A single irradiation dose of 20 Gy (160 kV, 1.15 Gy/min) was delivered to the whole thorax using an RS 2000Pro Biological Research Irradiator (Rad Source Technologies, Suwanee, GA, USA). Non-irradiated parts of the mice were shielded with 2 cm thick lead.

C. Treatment with GSN Antibody

The GSN antibody and control IgG were kindly supplied by the Department of Immunology of Soochow University. The GSN antibody used in this study was a polyclonal antibody. Half an hour before the 20 Gy thoracic irradiation, mice were injected with 50 µg GSN antibodies or IgG as control through the tail vein.

D. Measurement of GSN Levels

Mice were sacrificed at 6, 12, 24, 48, 72, 96, and 120 hours after irradiation. Serum samples were collected and stored at −80°C until use. Bronchoalveolar lavage (BAL) of the lungs was performed by lavaging three times with 0.5 mL phosphate-buffered saline (PBS) containing protease inhibitors. Recovery of fluid exceeded 90%. The lavage fluid was spun at 1500 rpm for 10 min and the supernatants were stored at −80°C. The levels of GSN in plasma and BAL fluid were measured by ELISA kit (Yuan Ye Biotechnology, China).

E. RT-PCR

Total RNA was extracted from approximately 100 mg of lung tissues using 1 mL of RNAiso reagent (TAKARA, Japan). RNA was precipitated with isopropanol and dissolved in diethyl pyrocarbonate-treated distilled water. cDNA was synthesized using M-MuLV reverse transcriptase (New England Biolabs, Ipswich, MA, USA) with oligo dT-adaptor primers. PCR was performed using Taq DNA polymerase (New England Biolabs) with the following primers: β-actin, sense: 5 -TGCGTGACATTAAGGAGAAG-3 , antisense: 5 -CTGCATCCTGTCGGCAATG-3 ; GSN, sense: 5 -AAAACTCGAGCCACCATGGCTCCGTACCGCTCTTC-3 , antisense: 5 -AAAATCTAGATCAGGCAGCCAGCTCAGC-3 .

F. Assessment of Lung Damage

After centrifugation of the BAL fluid, the cell pellets were resuspended in 1 mL PBS and stained with Wright' s-Giemsa staining solution. The absolute numbers of leukocytes in BAL fluid were counted using a hemocytometer. Protein concentration of BAL fluid was measured using a BCA protein assay kit (Beyotime, China).

One month after radiation treatment, lung tissues were removed and immediately fixed in 10% neutral-buffered formalin. The lungs were processed for conventional paraffin embedding, stained with hematoxylin-eosin (HE), and then evaluated under light microscopy.

G. Assessment of Oxidative Damage

Superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration were determined spectrophotometrically using their corresponding diagnostic reagent kits (Jiancheng Bioengineering, China) according to the manufacturer' s instructions.

H. Statistical Analyses

All results are expressed as mean $\pm$ SD. Differences in GSN levels and lung injury parameters were determined using one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

III. Results

A. Thoracic Irradiation Alters GSN Levels in Plasma and BAL Fluid

Plasma samples and BAL fluid from the control and IR groups were collected for measuring GSN concentration. As shown in Figure 1: see original paper, GSN levels significantly declined within 72 hours after irradiation and gradually recovered to basal levels after 120 hours. Meanwhile, GSN concentration in the BAL fluid increased slightly within 120 hours (Figure 1: see original paper).

GSN mRNA abundance declined significantly after irradiation, peaked at 24 hours, and then started to increase after 48 hours (Figure 1: see original paper). Our previous data also showed the same time-dependent changes of GSN protein levels as the mRNA levels in the lung tissues [19]. These results suggest GSN may be involved in the pathogenesis of radiation-induced lung injury.

B. Administration of GSN Antibody Exacerbates Radiation-Induced Lung Injury

GSN antibody was administered to Balb/c mice by intravenous injection. Half an hour after injection, the animals received 20 Gy whole-thorax irradiation. Histological examination of lung sections from mice 30 days after irradiation revealed severe diffuse congestion, focal alveolar hemorrhage, thickening of alveolar septa, and infiltration of inflammatory cells ([Figure 2: see original paper]). The IgG group showed equivalent grade of lung injury compared with the IR group. In addition, mice injected with GSN antibody showed more vascular congestion, alveolar hemorrhage, and leukocyte infiltration compared with mice treated with control IgG.

As shown in [Figure 3: see original paper], protein concentrations in BAL fluid, which indicate pulmonary vascular permeability, increased after thoracic irradiation. Protein levels peaked at 24 hours and decreased at 48 hours. However, pretreatment with GSN antibody greatly increased protein concentration in BAL fluid and maintained high levels at 48 hours. These results indicate that pretreatment with GSN antibody is able to exacerbate radiation-induced lung injury.

C. Pretreatment with GSN Antibody Increases Leukocyte Infiltration in Irradiated Lung

[Figure 4: see original paper] shows typical images of differential leukocyte counts in BAL fluid of mice after 20 Gy thoracic irradiation. There were more leukocytes in irradiated mice injected with GSN antibody than in the control group (injected with IgG). Leukocyte numbers were quantified ([Figure 5: see original paper]). The results showed that IR treatment increased leukocyte infiltration in BAL fluid, with the leukocyte number in BAL fluid of the anti-GSN group 24 hours after irradiation being three-fold higher than the control.

D. GSN Antibody Pretreatment Aggravates Oxidative Damage Induced by Irradiation

Three days after thoracic irradiation, SOD activity and MDA concentration in plasma and BAL fluid were measured. As shown in [Figure 6: see original paper], SOD activity in plasma decreased by 35% in irradiated mice injected with GSN antibody compared with the IgG control. However, there was no statistical significance in SOD activity in the BAL fluid. MDA concentration was about 1.5-fold higher in plasma and 1.9-fold higher in BAL fluid than in the IgG control.

IV. Discussion

Radiation-induced lung injury, characterized by acute pulmonary inflammation and irreversible pulmonary fibrosis, has been considered a major dose-limiting factor for radiation therapy of thoracic malignancies [1, 2]. The roles of GSN in radiation-induced pneumonitis were investigated. Mice pretreated with GSN antibody were subjected to 20 Gy thoracic irradiation. GSN levels in plasma significantly declined and gradually recovered to basal levels 120 hours after irradiation. GSN concentration in BAL fluid slightly increased within 120 hours. Meanwhile, GSN mRNA in lung tissues declined significantly, peaked at 24 hours, and then started to increase. The time-dependent changes of GSN protein levels in lung tissues were the same as the mRNA levels [19]. These changes in GSN levels indicated that GSN may play a critically important role in radiation-induced lung injury. Our data revealed deteriorative effects of GSN antibody on radiation-induced lung inflammation, suggesting GSN may protect mice against radiation-induced lung injury.

GSN is widely distributed in mammalian and non-mammalian animals, and the effects of GSN are rapid, stoichiometric, and highly efficient. There is evidence that GSN may lead to new considerations of this protein as a potential biomarker and/or therapeutic target [3–5]. Studies also revealed that GSN binding to circulating actin and inflammatory mediators could prevent damage in diverse states of acute insults, such as hepatic failure, trauma, myonecrosis, and sepsis [7–9, 20, 21]. GSN might be cleaved by proteases involved in epithelial remodeling that are expressed in airways, which have been found increased in the lungs in inflammatory lung diseases, including radiation-induced lung injury [5, 16, 18]. Our results showed the time-dependent changes in GSN levels in plasma, BAL fluid, and lung tissues in mice after thoracic irradiation.

It was reported that GSN exhibited strong positive staining within areas of the bronchial and alveolar epithelium and could be released by epithelial cells into the airways [5, 16, 18]. Therefore, it is hypothesized that GSN is involved in the pathogenesis of radiation-induced lung injury in mice.

In the present study, GSN antibody was administered to mice before thoracic irradiation to suppress GSN functionality. Histological examination displayed aggravated radiation-induced lung injury in mice pretreated with GSN antibody. Accordingly, it revealed the deteriorative effects of GSN antibody on pulmonary vascular permeability, inflammatory cell recruitment, and oxidative stress.

Thoracic irradiation may cause cellular damage and subsequent release of intracellular actin in the early period. Increased levels of circulating actin were considered a harmful factor for microcirculation. As an actin-scavenging protein, GSN could counteract actin toxicity when actin is released into the extracellular space. Plasma levels of GSN were significantly decreased 72 hours post-irradiation in mice, suggesting clearance of circulating actin by plasma GSN. To some extent, the degree of plasma GSN depletion should reflect the degree of lung injury. Intravenous infusion of GSN prevented burn-induced pulmonary microvascular dysfunction [17]. Administration of recombinant human GSN can diminish the acute inflammatory response of hyperoxic lung injury [13]. Thus, GSN levels may provide early evidence of evolving lung injury and an innovative diagnostic modality for acute lung injury. In the later period, due to high expression of GSN, large amounts of GSN were secreted into microcirculation from tissues and cells or peripheral GSN consumption reduced, thus plasma GSN levels quickly recovered to normal.

There was a large number of leukocyte infiltrations in the lungs of mice within 48 hours after thoracic irradiation. The leukocyte number in BAL fluid 24 hours after irradiation of mice treated with GSN antibody was three-fold higher than that of IgG-treated mice. GSN antibody injection resulted in significantly increased leukocyte infiltration. GSN is able to bind and inhibit bioactive inflammatory mediators, thus attenuating the inflammatory response. On the other hand, GSN deficiency not only contributed to impaired cytoskeletal rearrangement but also resulted in the development of increased pulmonary vascular permeability [5, 15]. Through cytospin preparations and histological examina-

tion, significantly more leukocytes were observed in irradiated mice injected with anti-GSN antibody compared with injection with control IgG.

Radiation results in the production of free oxygen radicals that likely contribute to subtle but critical cell injury in the lung very early after thoracic exposure [22]. From a structural point of view, GSN has some antioxidant potential [3, 5]. SOD activity and MDA concentration in plasma and BAL fluid were detected three days after thoracic irradiation. These data showed more oxidative damage in mice pretreated with GSN antibody, indicating GSN may play an important role in scavenging free oxygen radicals induced by thoracic irradiation.

V. Conclusion

The role of GSN in radiation-induced lung injury was investigated in Balb/c mice. GSN levels in plasma, BAL fluid, and lung tissues could be altered by thoracic irradiation. Pretreatment with GSN antibody aggravates radiation-induced pneumonitis, evidenced by increased lung inflammation, vascular permeability, and infiltration of leukocytes. GSN antibody also induced more oxidative damage from ionizing radiation. Thus, it is concluded that GSN may serve a protective role against radiation-induced lung injury under some circumstances.

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Note: Figure translations are in progress. See original paper for figures.