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Partial volume rat lung irradiation: The protective/mitigating effects of Eukarion-189, a superoxide dismutase–catalase mimetic

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Abstract

***Background and purpose*: The purpose of the current study was to elucidate the protective/mitigating effects of a SOD-catalase mimetic, Eukarion-189 (EUK-189), on DNA damage in rat lung following irradiation. The particular focus of these studies was the efficacy of EUK-189 when given after irradiation (mitigation).**

***Patients and methods*: We exposed whole or lower lungs of female Sprague–Dawley rats to doses ranging from 10 to 20.5 Gray (Gy) of ^{60}Co gamma rays. Animals in the EUK-189 treated groups received 2 or 30 mg/kg intraperitoneally (i.p.) at various times postirradiation (PI). A micronucleus assay was used to examine DNA damage at various times up to 16 weeks PI.**

***Results*: Our results indicated that EUK-189 administration after irradiation is effective at reducing micronucleus formation in lung fibroblasts at various times following radiation exposure. Treatment with EUK-189 in the first 3 days after thoracic irradiation did not, however, modify the dose required to cause severe morbidity at 2–3 months after irradiation.**

***Conclusions*: The protection produced when Eukarion-189 was given shortly after irradiation suggests that DNA damage observed in the lung may be caused by chronic production of ROS induced by a chronic inflammatory response initiated by the radiation treatment. We speculate that our failure to observe protection against severe morbidity at 2–3 months may be because our treatment regime only blocked the initial wave of ROS production and that treatment needs to be more prolonged to suppress the effects of a chronic inflammatory response.**
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Radiotherapy is an essential mode of treatment for nearly half of all cancer patients. Five to twenty percent of the patients who receive treatment in the thoracic region are at risk of developing radiation pneumonitis and/or fibrosis [24]. Managing normal tissue complications in the lung is therefore relevant to the planning and treatment of various neoplastic diseases, such as lung and breast cancer, Hodgkin's disease, and various lymphomas, where the lung invariably receives significant doses of radiation [1,12,44]. The ability to deliver tumoricidal doses while preserving the integrity of the surrounding normal tissue is critical to successful treatment with ionizing radiation. Thus, developing an in depth understanding of the mechanisms associated with radiation-induced normal lung damage is imperative.

The clinical manifestations of radiation damage in lung occur approximately 2 months after exposure and can continue to develop throughout the following years. Radiation pneumonitis is an acute inflammatory reaction that develops within the first 2–3 months postirradiation and is defined by edema resulting in abnormal chest X-rays and causing a shortness in breath, dyspnea, cough, and occasionally mild fever. Symptoms of radiation fibrosis can initiate 6 months to a year after pulmonary irradiation. Patients with radiation-induced lung fibrosis tend to have severe physiologic abnormalities and chronic respiratory failure [24]. The lungs become rigid, as a result of excess collagen deposition, stimulated by profibrotic cytokines and growth factors such as transforming growth factor-β (TGF-β) and platelet derived growth factor (PDGF) [11,37].

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Recent data suggest that pneumonitis and fibrosis may arise as a result of the interaction of a cycle of chronic inflammation and oxidative damage initiated by radiation damage in the lung [16,20,34]. A complex, integrated system of signals instruct pulmonary and inflammatory cells to react to the initial molecular injury. This response includes cell death, the production of reactive oxygen species (ROS), alterations in gene expression, and the production of cytokines [39,43]. A few key players, including alveolar macrophages, lung fibroblasts, and type II pneumocytes, participate in this signaling and interact via intercellular communication mediated by specific cytokines and growth factors [32,36]. Many studies suggest that temporal patterns of inflammatory cell and cytokine expression, which are initiated within hours after radiation, cause the generation of further ROS and are critical to the development of chronic inflammation and the progression of tissue deficits [14,15,22,33,35,37].

It is widely accepted that the dose, volume, fraction size, and use of chemotherapeutic agents all contribute to the degree of biological damage that can result following ionizing radiation [13]. Differential volume effects in radiation-induced lung damage have been demonstrated in a number of animal studies [19–21,26,39–41,45] and recent work has demonstrated that heart irradiation can also enhance the effects of irradiation on early and late lung damage [26,42]. Previous studies in our lab [19,20] have demonstrated a significant loco-regional volume effect on early DNA damage, measured by micronuclei formation, in pulmonary fibroblasts. Greater DNA damage was observed in-field upon irradiating the lung base compared with that of the apex. Furthermore, significant DNA damage was observed in the shielded upper lung after lower lung irradiation, which was not evident in the lower lung after apex irradiation. These results suggested that a mechanism exists which can generate damage within the entire organ after partial volume irradiation. This premise was further examined by administering superoxide dismutase (MnSOD, CuZnSOD) to the animals immediately prior to radiation. These compounds significantly lowered the out-of-field DNA damage observed in the upper lung suggesting a role of oxidative damage.

A link between oxidative stress and radiation damage to lung is becoming clearer with an increasing number of studies focusing on radioprotection [4,8,18,28,29,31,43]. Our current study examines the protective effects of a SOD-catalase mimetic, Eukarion-189 (EUK-189), on early and late DNA damage. EUK-189 is a low molecular weight, Mn-containing coordination complex that has been shown to protect cells in intracellular or extracellular injury models [25,27]. Thus, we hypothesized that, given prior to radiation, it would be effective in reducing ROS created within the cell as a direct result of radiation as well as potentially reducing the ROS burden generated as a part of the inflammatory response created extracellularly and subsequent to the direct radiation insult. We wished to investigate the protective capacity of this compound when administered postirradiation (PI) to discern its effects on direct and indirect molecular damage created in- and out-of-field.

Materials and methods

Irradiation procedure

Seven to eight weeks old female Sprague–Dawley rats, weighing 180–220 g at the time of irradiation, were used in all of the experiments. The animals were housed and treated according to guidelines approved by the Canadian Council on Animal Care.

The whole lung or the lower half of the lung (70% of total lung volume) were exposed to single doses ranging from 10 to 20.5 Gy of ^{60}Co gamma irradiation (dose rate ~0.57 Gy/min). Quantification of the volume of lung irradiated and the effect of the shielding block position were established previously [19,20]. Immediately prior to irradiation the animals were placed in holding containers and anaesthetized by halothane inhalation. Diagnostic X-rays of the animals were taken to determine the position of the lungs relative to fixed lead markers on the holding containers. Lead shielding blocks, 10 cm in thickness, were used to delineate the radiation field, ensuring that adjacent tissues were not exposed. For whole lung irradiation, shielding blocks were separated by 3 cm, the total length of the lung, from the second rib insertion, specifying the cranial boundary, to below the dome of the diaphragm, specifying the caudal boundary. For lower lung irradiation the lead block was placed 1.5 cm below the second rib insertion, shielding the upper lung (30% of the total lung volume) as described previously [19].

Eukarion-189 treatment

The superoxide dismutase (SOD)-catalase mimetic, EUK-189, prepared as described previously [7], was generously supplied by Eukarion Incorporated (now part of Proteome Systems, Inc., Woburn, MA, USA). For the micronucleus assay animals were randomly assigned to irradiation and EUK-189 treatment or irradiation only groups. The animals in the EUK-189 treatment groups received intraperitoneal (i.p.) injections of 2 mg/kg body weight or 30 mg/kg body weight. EUK-189, and a related compound EUK-134, administered in this dose range attenuates neurological damage caused by oxidative stress in mice [17,25] and rats [27]. The compounds were administered either before irradiation (30 min) or after irradiation (5 min, 1, 2 h, 1, 2, 3 days, or 1, 2 weeks) for the micronucleus assay.

Micronucleus assay

A well-established cytokinesis-block micronucleus assay [19,20] was used to assess genomic damage, in the form of DNA double strand breaks, created post radiation exposure. Briefly, alpha MEM media supplemented with antibiotics (Sigma–Aldrich Canada Ltd, Oakville, Ont., Canada) was perfused through the right ventricle of the hearts of deeply anaesthetized animals to remove as much blood in the lungs as possible. Lung quadrants were then removed aseptically and a section of the mid lung (0.5 cm on either side of the field edge) was discarded to account for any uncertainty in the field edge in the lower lung irradiations [19]. This section of the mid lung was also removed after the whole lung irradiations, to maintain consistency between the lower lung and whole lung analyses. The lungs were minced

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