DIETARY FLAXSEED AS COUNTERMEASURE TO LUNG DAMAGE RESULTING FROM REPEATED EXPOSURE TO RADIATION AND HYPEROXIA ASSOCIATED WITH SPACE EXPLORATION

Ralph A. Pietrofesa1, Floyd Dukes1, Sonia Tyagi1, Evgenia Arguiri1, Charalambos C. Solomides2, and Melpo Christofidou-Solomidou1

1Departments of Medicine, Pulmonary, Allergy, and Critical Care Division, University of Pennsylvania Medical Center, Philadelphia, PA 19104 2Department of Pathology, Jefferson University Hospital, Philadelphia, PA 19140

BACKGROUND
Spaceflight missions may require crewmembers to conduct extravehicular activities (EVA) for repair, maintenance or scientific purposes. EVAs may last for a few hours (5-8 hours) and must not exceed 24 hours weekly per crew member. During each EVA, crewmembers are subjected to a number of challenges that may present a threat to their health and therefore pose a limitation to the success of the mission. One such challenge is the exposure to lung damaging hyperoxic conditions (>95% O2) as well as to low levels of total body cosmic/galactic radiation. Currently, no studies have addressed the effects of this double-hit hyperoxia and radiation exposure. Therefore, our objectives were to: 1) characterize the effects of a double-hit challenge on lung tissue using a novel murine model of repeated exposure to low level radiation and hyperoxia and 2) test the usefulness of FS as a countermeasure to lung damage resulting from this double-hit model, given the lung protective properties of dietary flaxseed (FS) in mouse models of acute and chronic lung injury.

METHODS
We first designed a novel murine model of repeated exposure to radiation and hyperoxia. Mouse cohorts (n=10/group) were exposed to: a) normoxia; b) >95% O2 (O2); c) 0.25Gy gamma radiation (RAD); or d) a combination of O2 and RAD (O2+RAD). Challenge (O2, RAD or O2+RAD) was given 3 times per week whereby 8-hour hyperoxia was spanned by normoxic intervals (ambient air). Lungs were evaluated after 1, 2, or 4 weeks of exposure for oxidative damage, inflammation, injury and fibrosis. Systemic neutrophil (PMN) activation was also evaluated using flow cytometry (FACS) to detect PMN activation, myeloperoxidase (MPO), platelet activation (CD41). For our second objective, mouse cohorts (n=5/group) were pre-fed diets containing either 10% FS or an isocaloric control, 0% FS diet for 3 weeks and exposed to similar challenges. Additionally, gene expression changes in lung tissues were also evaluated on select genes such as heme oxygenase 1 (HO-1) and NADPH: quinone oxidoreductase 1 (NQO-1), associated with antioxidant response and inflammation using qRT-PCR.

RESULTS
All treatment challenges (O2, RAD, O2+RAD) resulted in significant mouse lung injury and inflammation (p<0.05) evidenced by increased bronchoalveolar lavage (BAL) protein levels and significant inflammatory cell infiltration (p<0.003), respectively. Specifically, O2 and O2+RAD-exposed mice had higher oxidative tissue damage (p<0.01), while after just 1 wk of treatment, elevated lung tissue fibrosis was detected (p<0.04). O2 and O2+RAD-exposed mice had higher lung malondialdehyde (MDA) levels (p<0.01), indicative of oxidative tissue damage. After a single cycle of exposure, all groups had elevated blood-derived neutrophil activation markers such as CD18, CD41, and MPO. Having defined the kinetics of tissue damage and determined that 2 weeks is sufficient to induce significant oxidative and inflammatory changes in lung, we proceeded to test FS as a countermeasure to damage.

For this, as anticipated, mice fed 0% FS developed significant lung injury and inflammation across all challenges, as evidenced by BAL neutrophils (p<0.003) and increased BAL protein levels, as well as increased oxidative tissue damage (p=0.008), whereas 10% FS ameliorated all adverse parameters. Lung hydroxyproline content also increased in 0% FS-fed mice exposed to RAD and O2+RAD (p<0.001) but abrogated in 10% FS-fed mice. Genes associated with inflammation, such as tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α), and antioxidant enzymes, such as HO-1 and NQO-1, were significantly upregulated in mice fed 0% FS and exposed to O2+RAD. However mice fed 10% FS had inflammatory and antioxidant gene expression levels comparable to baseline values, suggesting the anti-inflammatory and antioxidant potential of dietary FS.

CONCLUSION
We have characterized a novel murine model of repeated radiation and hyperoxia exposure. We identified EVA-related lung tissue changes associated with inflammation, fibrosis and oxidative tissue damage. We have also identified dietary FS as a potent countermeasure against the lung damaging effects of these oxidative challenges. In conclusion, dietary FS may represent a potential supplement that can be used to abrogate the detrimental health effects of radiation and hyperoxia exposure associated with space exploration.