Novel Double-Hit In Vitro Model of Oxidative Damage Associated with Cycling Exposure to Radiation and Hyperoxia Relevant To Space Travel

Ralph A. Pietrofesa1, Evguenia 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

Pre-breathe protocols in preparation for extravehicular activities (EVA) entail 100% hyperoxia exposure that may last for a few hours (5-8 hours), and may be repeated 2-3 times weekly. Each EVA is associated with additional challenges such as low levels of total body cosmic/galactic radiation exposure that may present a threat to crewmember health and therefore, pose a threat to the success of the mission. Repeat cycling changes of oxygen levels may pose an added threat. Using a murine model of repeated hyperoxia and radiation exposure (double-hit), we have previously observed significant early and chronic lung inflammation, injury, and oxidative tissue damage.1 In order to explore the potential mechanism(s) behind the observed lung tissue damage, we developed a novel in vitro model of hyperoxia and radiation exposure in the context of evaluating countermeasures to lung cell damage associated with space flight. Our objective was to characterize the effects of repeated single and double-hit challenge on lung cells using a novel in vitro model of repeated exposure to radiation and hyperoxia. This is the first study of its kind evaluating lung cell damage relevant to space exploration in an in vitro model system.

METHODS

We have previously designed a novel murine model of repeated exposure to total body irradiation (TBI) and hyperoxia.1 Thus, we developed in accordance to the in vivo experimental design, an in vitro model of double-hit exposure in which non-tumorigenic, normal murine alveolar Type II epithelial cells (C10) were exposed to challenge conditions of: a) normoxia; b) >95% O2 (O2); c) 0.25Gy single fraction gamma radiation (IR); or d) a combination of O2 and IR (O2+IR). Lung cells were plated in coated 6cm petri dishes and enclosed in specially-designed O-ring sealed, leak-proof aluminum chambers that allow full exposure to gamma rays. The atmosphere inside the chambers was changed to the desired oxygen content through a system of valves and manifold. Chambers were kept at 37°C at all times. Cells were exposed to hyperoxic conditions for 8 hours followed by normoxia (ambient air containing 5% CO2) for 16 hours representing 1 cycle of exposure. Cells were harvested after 1 cycle (24 hours), 2 cycles (48 hours) or 3 cycles (72 hours) of exposure at which time apoptosis (determined by quantification of apoptotic bodies), DNA fragmentation (measured by COMET analysis), and cell survival (Crystal Violet-stained cells) were determined. Additionally, levels of key proteins implicated in genotoxic stress (cleaved-Poly (ADP-ribose) polymerase (cleaved-PARP), growth arrest and DNA damage (GADD45), cell cycle progression (cyclin-dependent kinase 1 (CDK1) and cyclin B1, and p21) were determined by Western blot analysis.

RESULTS

Combined radiation and hyperoxia exposure resulted in significant oxidative, nitrosative, and genotoxic lung damage in our murine model of repeated exposure.1 By further exploring these observations in an in vitro model of relevant combined radiation and hyperoxia exposure, we found evidence of genotoxic stress-induced lung cell death resulting from exposure to challenge conditions. Specifically, after one cycle of exposure to O2, IR, or O2+IR, DNA damage as evidenced by COMET increased 2.3-, 1.8-, and 2.5-fold from untreated cells, respectively. Cellular apoptosis was significantly (p<0.05) increased due to combined effects of radiation and hyperoxia exposure in terms of apoptotic bodies, detected in 8.5, 7.9, and 7.9% of cells exposed to O2+IR after 1, 2, and 4 cycles of exposure (by comparison, control cells exhibit 3.0, 2.4, and 1.8% apoptosis). Similarly, lung cells exposed to combination radiation and hyperoxia displayed significantly (p<0.05) decreased survival. Relative to untreated cells, cells exposed to O2+IR had 36, 65, and 82% decreased overall survival after 1, 2, and 3 cycles of exposure, respectively. Both cleaved-PARP and GADD45, protein markers of DNA damage and genotoxic stress, were significantly (p<0.05) elevated after one cycle of exposure and persisted, most notably in cells exposed to O2+IR. CDK inhibitor, p21, was significantly upregulated in cells exposed to the combined challenge (protein levels equivalent to 16-, 17-, and 18-fold change over untreated cells) after 1, 2, and 3 cycles of exposure. This correlated with an observed 91, 54, and 79% decrease in CDK1 and 92, 69, and 81% decrease in cyclin B1 relative to unexposed, control cells.

CONCLUSION

Our in vitro findings suggest that the observed in vivo lung damage due to double-hit exposure is associated with increased DNA damage, cell cycle arrest, and subsequent apoptosis, as well as overall cell death. These findings were observed in cells exposed to individual challenges after all designated time points and were most notable in cells exposed to the combined radiation plus hyperoxia exposure. Importantly, these findings support the use of an in vitro model system of radiation and hyperoxia exposure relevant to space travel and provide a potential mechanism behind our previous in vivo observations. These results are well correlated with the observed oxidative tissue damage that was observed across all parameters that we measured; in addition to being significantly exacerbated by the combined exposure to O2 and IR. This data will provide useful information in the design of countermeasures to oxidative lung damage associated with space exploration.

Reference