ADAPTATION TO SUPRAPHYSIOLOGIC ROOM AIR OXYGEN CHANGES THE EFFECTS OF NITRIC OXIDE ON A549 LUNG CELLS IN VITRO

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Abstract

B320 533.89

Airway epithelial cells are exposed to some of the highest O_2 levels encountered *in vivo*. However, O_2 levels, even in the lungs, are far lower than room air (~10% vs 21% O_2, respectively). The culture and handling of airway cells in room air can alter the redox status of the cells, introducing artifact. Nitric oxide (NO), which can affect HIF-1alpha stabilization and bind cytochrome oxidase, has been shown to play multiple roles in the response of lung to hypoxic injury and inflammation. Here we explore whether pre-adaptation of the commonly used human lung epithelial cell line A549 to room air O_2 levels changes cellular responsiveness to NO. Using the Xvivo System to provide full-time control of O_2 and CO_2 during cell incubation as well as handling, we adapted A549 cells to 18% supraphysiologic O_2 (standard room air incubator with humidity and CO_2), physiologic O_2 (10%) or pathologic hypoxia (2% O_2). We assayed cellular responses to exposure to NO in standard plate-based cytotoxicity assays. We found that adaptation to different O_2 levels had a concentraction-dependent effect on the responses of A549 cells to NO. We concluded that it is critical to consider the O_2-adapted state of the cell prior to assay to avoid O_2 artifact.

Background

- **Room air incubators have much higher O_2 levels than tissues in vivo[1]**
- **Culture in room air can alter cells' redox capacity, introducing artifact[2]**
- **The O_2 history of the cell can change NO production and affect cell responses to NO[3]**
- **Inhaled NO can affect lung development[4]**

Objectives

- **Thoroughly adapt A549 human lung carcinoma cells to 2%, 10%, and 18% (standard room air incubator) O_2**
- **Assess if responses to gaseous NO are changed**

References

1. Wenger RH, Kurtcuoglu V, Scholz CC, Marti HH, Hoogewijs D: Frequently asked questions in hypoxia research. Hypoxia 2015, 3:35-43.
2. Kumar A, Dailey LA, Swedrowska M, Siow R, Mann GE, Vizcay-Barrena G, Arno M, Mudway IS, Forbes B: Quantifying the magnitude of the oxygen artefact inherent in culturing airway cells under atmospheric oxygen versus physiological levels. FEBS Lett 2016, 590(2):258.
3. Ball KA, Nelson AW, Foster DG, Poyton RO: Nitric oxide produced by cytochrome c oxidase helps stabilize HIF-1α in hypoxic mammalian cells. Biochemical and biophysical research communications 2012, 420(4):727-732.
4. Duong-Quy S, Hua-Huy T, Pham H, Tang X, Mercier J, Baud O, Dinh-Xuan A: Early inhaled nitric oxide at high dose enhances rat lung development after birth. Nitric Oxide 2014, 38:8-16.culturing airway cells under atmospheric oxygen versus physiological levels. FEBS Lett 2016, 590(2):258-269.

Experimental Design

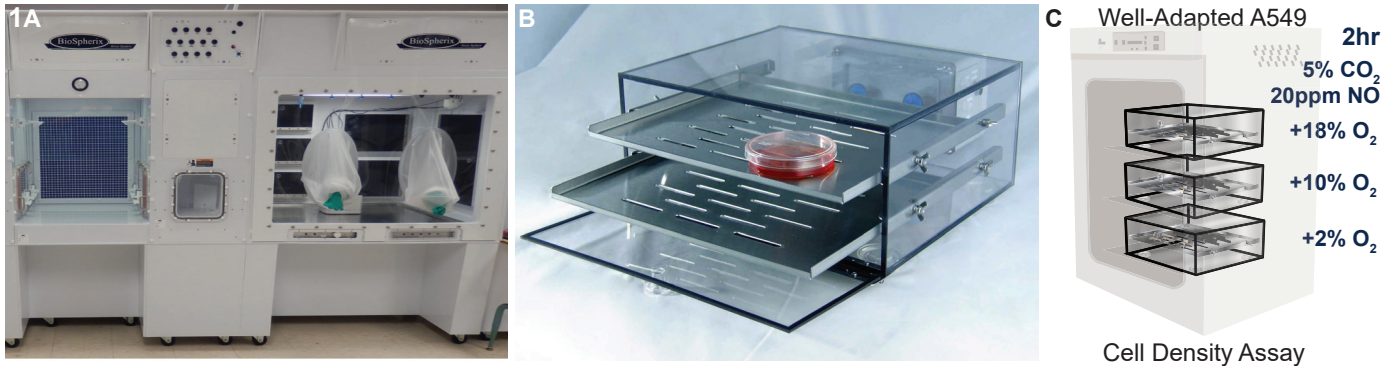

Figure 1. Experimental Design. (A) The Xvivo System was used to maintain A549 cells in constant conditions during cell handling as well as incubation. Incubators (black doors) opened only into a sealed cell handling space supplied with tanked, medical-grade gases. The processing chamber floor and air were heated and the air was continuously HEPA filtered. RPMI 1640 + 10% FBS + L-Glutamine (Gibco) with no antibiotics was pre-equilibrated to the proper O_2 level for at least 12 hr before use. A549 were plated (18 wells/sample/plate with the rest of the wells filled with PBS) in 96-well plates at 2500 cells/well O/N before 2hr exposure to 20ppm NO for in a standard CO_2 incubator subchamber (B) equipped with an OxyCycler GT4181N controller. (C) Schematic. Cells were returned to the Xvivo incubated 24, 48, or 72 hr before assessment for cell density using a standard crystal violet assay.

Results

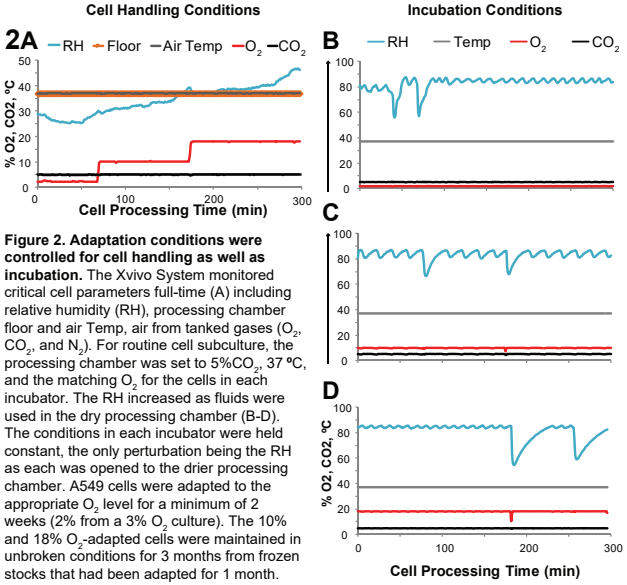

Figure 2. Adaptation conditions were controlled for cell handling as well as incubation. The Xvivo System monitored critical cell parameters full-time (A) including relative humidity (RH), processing chamber floor and air Temp, air from tanked gases (O_2, CO_2, and N_2). For routine cell subculture, the processing chamber was set to 5%CO_2, 37 °C, and the matching O_2 for the cells in each incubator. The RH increased as fluids were used in the dry processing chamber (B-D). The conditions in each incubator were held constant, the only perturbation being the RH as each was opened to the drier processing chamber. A549 cells were adapted to the appropriate O_2 level for a minimum of 2 weeks (2% from a 3% O_2 culture). The 10% and 18% O_2-adapted cells were maintained in unbroken conditions for 3 months from frozen stocks that had been adapted for 1 month.

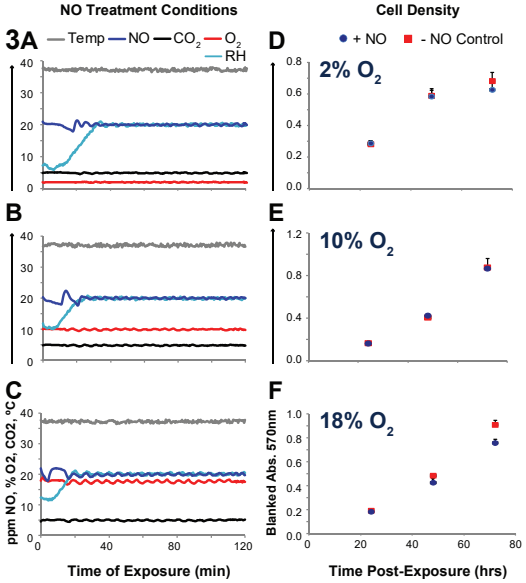

Figure 3. A549 Adapted to Room Air Incubation Were Affected More by Exposure to NO than A549 Incubated at Physiologic or Hypoxic Conditions. (A-C) A549 cells were exposed to 20ppm NO for 2 hr as well as their own O2 levels (2, 10 or 18% O_2). RH levels were kept low in the chamber to reduce NO2 and NO3 formation in the gas phase. Rising RH after adding the plates to the chamber drove gas adjustments in the first 20min of incubation. Cell density was assessed at 24, 48, and 72 hr after exposure (D-F). There was no evident effect of the NO exposure upon cells incubated at 2 or 10% O_2, but the cells maintained at room air incubator conditions (18% O_2) trended toward reduced growth over time. This suggests that different results can be obtained by incubation of cells at physiologically relevant O_2 levels as compared with supraphysiologic room air incubator oxygen levels.

Conclusions

Adaptation to room air oxygen can alter the responses of human lung cells to nitric oxide. Maintain cells at unbroken physiologic oxygen for more physiologically relevant results.