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Probing Real Time Redox Events in Human Airway Epithelial Cells Exposed to an Environmental Peroxide

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Air pollutants such as ozone, particulate matter, and secondary organic aerosols (SOA) induce intracellular oxidative stress via the generation of reactive oxygen species (ROS). While ROS play important roles in regulating signaling pathways, supra-physiological levels disrupt redox homeostasis and potentiate inappropriate oxidation of regulatory thiols. We examined the effect of isoprene hydroxy hydroperoxide (ISOPOOH), an environmentally derived peroxide that contributes to SOA, on the interplay between bioenergetics and intracellular redox status. We used live cell imaging of human airway epithelial cells (HAECs) expressing the genetically encoded ratiometric biosensors roGFP, iNAP1, and HyPER, to monitor changes in the glutathione redox potential (E_{GSH}), NADPH and H_2O_2, respectively. Non-cytotoxic exposure to ISOPOOH induced transient increases in E_{GSH} in HAECs that were markedly potentiated by glucose deprivation. ISOPOOH-induced changes in E_{GSH} were not driven by intracellular H_2O_2. Following ISOPOOH exposure, the addition of 1 mM glucose rapidly restored baseline E_{GSH} and reversed ISOPOOH-induced reductions in NADPH levels, while lower concentrations of glucose (30 uM) induced a bi-modal E_{GSH} recovery. Alternatively, the addition of the glycolytic inhibitor 2-deoxyglucose (2-DG) did not block recovery of NADPH levels nor E_{GSH} restoration. To impair the recovery of E_{GSH} and NADPH levels, we employed a lentiviral vector system to knockdown glucose-6-phosphate dehydrogenase (G6PD), a key enzyme involved in NADPH synthesis. The resulting G6PD knockdown (~50%) did not block glucose-mediated recovery of E_{GSH}, implicating that a partial knockdown of G6PD may not be sufficient to manipulate NADPH levels and thereby E_{GSH}. These findings underscore early mechanisms involved in the cellular response to ISOPOOH while providing a unique live view of the dynamic regulation of redox homeostasis in the human lung during exposure to environmental oxidants. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.