Abstract

Background: The toxicity of many compounds involves oxidative injury to cells. Direct assessment of mechanistic events involved in xenobiotic-induced oxidative stress is not easily achievable. Development of genetically-encoded probes designed for monitoring intracellular redox status represents a methodological advance with potential applications in toxicological studies.

Objective: Test the utility of redox sensors in monitoring intracellular redox status of toxicological studies involving xenobiotics.

Methods: roGFP2, a redox reporter of the intracellular GSH/GSSG ratio, was used to monitor the redox potential of cultured BEAS 2B cells undergoing exposure to 0.15 – 1.0 ppm O_3. Cells were imaged in real-time using a custom-built exposure system coupled to a confocal microscope. The roles of glutaredoxin 1 (Grx1), glutathione peroxidases (GPx), H_2O_2, and mitochondrial redox in transducing GSSG levels reported by roGFP in cells exposed to O_3 were examined.

Results: O_3 exposure induced dose- and time- dependant losses in intracellular reducing potential. Grx1-linked roGFP2 was observed to enhance the kinetics of O_3-induced loss of reducing potential while inhibition of endogenous Grx1 was shown to disrupt roGFP responses to oxidative insult. Selenite-induced GPx overexpression was observed to increase the rate of reducing potential losses. O_3-induced H_2O_2 production and mitochondrial dysfunction did not appear to initiate the losses in reducing potential reported by cytosolic roGFP2.

Conclusion: Exposure to O_3 induces a profound loss of reducing potential in airway epithelial cells that is indicative of an oxidant-dependent impairment of redox homeostasis. These studies demonstrate the utility of using genetically-encoded reporters in making reliable assessments of cells undergoing exposure to xenobiotics with strong oxidizing properties.