In Vitro Investigation of Integrated Stress Response Activation by 4-Hydroxynonenal

Chung, Jaein[1]; Rodriguez, Jessica R.[2]; Laskin, Debra L.[2]

[1]Dartmouth College, Hanover, NH
[2]Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ

Ground-level ozone is a harmful air pollutant associated with 300,000 deaths annually. Inhaled ozone reacts with airway epithelial membranes and pulmonary surfactant to generate lipid peroxidation products, including aldehyde 4-hydroxynonenal (4-HNE), which forms adducts with proteins and disrupts function. Although ozone-induced oxidative injury has been explored, the cellular pathways promoting recovery from this damage remain less understood. One promising mechanism is the integrated stress response (ISR). In response to cellular stress, various kinases phosphorylate eukaryotic translation initiation factor 2 alpha (eIF2α) to reduce global protein translation and selectively upregulate protective genes. Because the oxidative effects of 4-HNE can trigger ISR kinases, the ISR may be a protective mechanism following ozone exposure. To investigate this potential role, murine macrophages (RAW 264.7) and alveolar epithelial type 2 (MLE-15) cells were treated with 25µM 4-HNE for 3 hours. Protein was then isolated and examined through western blotting. Both RAW and MLE cells exhibited increased phosphorylation of eIF2α and increased expression of nuclear factor erythroid 2-related factor 2 (NRF2), an antioxidant transcription factor activated by ISR kinases. Additionally, 4-HNE increased prolyl-4-hydroxylase domain 3 (PHD3), a negative regulator of activating transcription factor 4 (ATF4) -mediated ISR signaling. The increase in PHD3 expression suggests that there may be ISR-mediated transcription independent of ATF4. Among antioxidant proteins, heme oxygenase-1 (HO-1) expression increased, whereas superoxide dismutase (SOD1) expression remained unchanged. These data demonstrate that 4-HNE activates the ISR to promote an antioxidant response, supporting a potential protective role of the ISR following ozone-induced oxidative stress. Supported by NIH grants ES004738, ES005022, R25ES020721, the Society of Toxicology, and the Air Pollution Educational and Research Grant (APERG) Scholarship Program.