Potential Role of Ferroptosis in Toxicity Induced by 9,10-Phenanthrenequinone in Human Lung Epithelial Calu-1 Cells

Author Block: Y. An\textsuperscript{1}, Y. Jan\textsuperscript{1}, D. E. Heck\textsuperscript{2}, D. L. Laskin\textsuperscript{1}, and J. D. Laskin\textsuperscript{1}. \textsuperscript{1}Rutgers, The State University of New Jersey, Piscataway, NJ; and \textsuperscript{2}New York Medical College, Valhalla, NY.

9,10-Phenanthrenequinone (9,10-PQ), which is a redox-active polycyclic aromatic hydrocarbon found in diesel exhaust particles and cigarette smoke, plays a crucial role in the adverse health effects triggered by air pollution. The toxicity of 9,10-PQ has been associated with the generation of reactive oxygen species (ROS) via the process of redox cycling. In the present studies, we investigated mechanisms of 9,10-PQ induced toxicity in human lung epithelial Calu-1 cells. 9,10-PQ treatment caused a time- and concentration-dependent decrease in cell viability. This was associated with a decrease in intracellular glutathione (GSH) and glutathione peroxidase (GPX) activity, 9,10-PQ caused an increase in the ratio of GSSG to GSH. 9,10-PQ also increased levels of intracellular ROS and lipid peroxidation products in a concentration- and time-dependent manner, as measured by the CM-H\textsubscript{2}DCFDA and BODIPY assays, respectively. These data indicate that 9,10-PQ caused oxidative stress in Calu-1 cells. N-acetyl cysteine (NAC) and β-mercaptoethanol (β-ME) pretreatment inhibited 9,10-PQ induced ROS generation and partially suppressed 9,10-PQ-induced cell death. Regulated cell death characterized by the iron-dependent accumulation of lipid peroxides, referred to as ferroptosis. Ferrostatin-1, a lipid ROS scavenger and specific inhibitor of ferroptosis, and deferoxamine mesylate (DFO), an iron (III) chelator, were found to decrease 9,10-PQ induced ROS production and protect Calu-1 cells from toxicity. In contrast, z-VAD-FMK, a pan-caspase inhibitor, had little or no effect on 9,10-PQ toxicity, indicating caspase-mediated apoptosis was not involved in the actions of 9,10-PQ. Taken together, these data indicated that ferroptosis is a critical cell death pathway induced by 9,10-PQ. Support: NIH grants AR055073, NS108956, ES004738, and ES005022.