mitochondrial Trx and glutathione under these conditions. Formation of acrolein-Trx1 adduct completely inhibits Trx1 activity, stimulates production of reactive oxygen species, and promotes monocytes adhesion to the endothelium. These results suggest that air pollution may contribute to atherosclerosis development by interfering with antioxidant and redox signaling of Trx1.

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ROLE OF OXIDANTS IN PM-INDUCED ANGIOTENSIN II RECEPTOR-ERK1/2 SIGNALING IN HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS

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Particulate matter (PM) exposure is associated with cardiovascular adverse effects, but the mechanisms are unclear. We showed previously that PM activated ERK1/2 in human pulmonary artery endothelial cells (HPAEC) and constricted pulmonary artery via angiotensin II receptor (AT1R). Since PM also enhances early production of reactive oxygen species (ROS), in this study, we determined the role of ROS in the AT1R-ERK1/2 signaling. HPAEC were treated with St. Louis urban particle (UP). ERK1/2 phosphorylation (pERK1/2) and H2O2 production were measured. UP and its soluble fraction dose-dependently increased pERK1/2 as early as 5 min after exposure while they increased H2O2 production after 40 min. H2O2 production was attenuated by losartan (an AT1R antagonist), SOD, PD58095 (a MAPK kinase inhibitor), catalase, diphenyliodonium (a flavoprotein inhibitor), sodium azide (a mitochondrial complex IV inhibitor) and deferroxamine, but only losartan and SOD inhibited pERK1/2. Other inhibitors of ROS-generating enzymes, including allopurinol, cimetidine, indomethacin, rotenone and antimycin, had no effects on either pERK1/2 or H2O2 production. UP-induced constriction of rat pulmonary artery was inhibited by losartan, SOD and PD58095. Exogenous H2O2 did not activate ERK1/2, but induced vasoconstriction in perfused lungs. These results indicate H2O2 production induced by PM was mediated in part by activation of AT1R-ERK1/2 and the sources for H2O2 may be NAD(P)H oxidases. Also, superoxide production preceeded ERK1/2 activation. ROS-mediated pulmonary vasoconstriction may be an important mechanism for PM-induced cardiovascular health effects, especially in patients with right heart failure (This abstract does not reflect EPA policy).

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DIESEL EXHAUST PARTICLES ENHANCE INFLUENZA VIRUS INFECTIONS VIA OXIDATIVE STRESS

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Diesel exhaust (DE) is an important contributor to air pollution induced adverse health effects, such as increased allergic airway diseases. Many of the adverse effects caused by exposure to DE particles (DEP) are mediated by oxidative stress, possibly through polyaromatic hydrocarbons (PAH) adsorbed onto the surface of DEP. In this study we examined the effects of DEP on influenza infections and the role of oxidative stress in these responses. Differentiated human bronchial and nasal epithelial cells as well as A549 cells, were exposed to DEP for 2 hours and subsequently infected with Influenza A. In all three epithelial cell models exposure to DEP enhanced influenza virus infection, which was not caused by decreased antiviral mediator production, such as interferon beta or MxA. Immunofluorescence analysis showed that the number of cells infected with influenza was enhanced after exposure to DEP, suggesting that attachment and uptake of the virus was enhanced by DEP. Exposure to DEP induced oxidative stress as measured by enhanced protein carbonyl levels, which were decreased by addition of reduced glutathione (GSH). Similarly, addition of GSH also reversed the effect of DEP on the number of influenza infected respiratory epithelial cells, suggesting that DEP-enhanced influenza infectivity is mediated by oxidative stress. Surfactant protein D (SP-D) is secreted by respiratory epithelial cells and an important mediator of innate immune defenses against influenza through its ability to bind and thus inhibit the infectivity of influenza. DEP caused oxidation of SP-D, which could alter its ability to bind and inhibit influenza infectivity. Taken together these data suggest that DEP-induced oxidative stress enhances influenza infections of human respiratory epithelial cells possibly by oxidatively modifying the function of important innate immune defense mediators.

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EXTRACELLULAR ANTIOXIDANTS AND DESFERRIOXAMINE INFLUENCE $^{\bullet}NO_2$-MEDIATED PROTEIN MODIFICATIONS

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Nitrogen dioxide ($^{\bullet}NO_2$) exposure results from direct inhalation, oxidation of $^{\bullet}NO$, decomposition of peroxynitrite ($ONOO^-$), and via myeloperoxidase (MPO) and initiates oxidative stress, epithelial damage, and inflammation. Lung epithelial exposures are governed, in part, by $^{\bullet}NO_2$ diffusion and reaction within the epithelial lining fluid (ELF) which yields secondary reactive species. Using a lung surface compartmentation model, HBE1 cells or red cell membranes (RCM) were exposed to $^{\bullet}NO_2$ (5 ppm, 2 hrs), while intermittently covered by a chemically defined aqueous film, and protein oxidation (carbonyls), nitration (3-nitrotyrosine), and tyrosine phosphorylation were assessed. Without reactive substrates, $^{\bullet}NO_2$ induced robust HBE1 nitration and phosphorylation while oxidation was marginally increased. Extracellular GSH increased exposure-related oxidation, decreased nitration, but did not affect phosphorylation. Protein modifications (+/- GSH) were unaffected by iron chelation with DTPA; however desferrioxamine (DFX) inhibited nitration and perhaps enhanced GSH-mediated oxidation. In the RCM model, protein nitration was iron-independent and DFX-inhibitable during gas phase or $MPO/H_2O_2/NO_2^-$-mediated $^{\bullet}NO_2$ exposures. Based on measures of relative reactivity, our data suggest that DFX inhibits nitration by reducing the tyrosyl radical rather than directly scavenging $^{\bullet}NO_2$ or chelating iron. Furthermore, the extracellular chemistry between $^{\bullet}NO_2$ and ELF constituents generates nitroxidative stress resulting in protein oxidation, nitration, and phosphorylation that likely contribute to $^{\bullet}NO_2$ induced pathophysiologic sequelae observed in vivo. (HL 54696)

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PULMONARY CARBON NANOTUBE EXPOSURE AND OXIDATIVE STATUS IN VASCULAR SYSTEM

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Cardiovascular diseases, which in majority of cases stem from atherosclerosis, continue to be the principal cause of death in the United States. In addition to personal factors like hyperlipidemia and obesity, some environmental factors including cigarette smoking and air pollution, have been associated with cardiovascular diseases. Engineered nanosized particles, such as

carbon nanotubes (CNT), are new materials of emerging technological importance in different industries. The unique physical characteristics of these particles raise concerns that they may have not only pulmonary toxicity but also be associated with extra-pulmonary toxicity including heart and vessels. In the present study, we hypothesized that CNT pulmonary exposures can induce oxidative changes in the vasculature related to atherogenesis. C57BL/6 mice were exposed to CNT in doses (0.5; 1; 2 mg/kg) by single intra-pharyngeal installation and the mice were sacrificed at different time points (1; 7; 28; 56 days) after the exposure (the experimental settings have been related to pulmonary toxicity). Genomic DNA was isolated from the aortas of these mice and the oxidative effects were measured by extra long quantitative PCR of mitochondrial (mt)DNA. We found that CNT exposures are associated with dose-dependent aortic mtDNA damage at day 7, 28 and 56 after exposure. MtDNA damage might be a direct result from CNT which penetrate to the circulation or an indirect result of the lung inflammation. The direct oxidative potential of CNT was evaluated in vitro by measuring the mtDNA damage in human aortic endothelial cells (HAEC) as well as the oxidation of low density lipoproteins (LDL) in the presence of HAEC. CNT dose dependently induced oxidative modifications in this in vitro system. In conclusion, CNT induces direct or indirect oxidative effects which might be predisposing factors for atherogenesis.

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H_2O_2 MEDIATES INFLAMMATORY AND TOXIC RESPONSES TO AMBIENT AIR PARTICLES IN PRIMARY LUNG EPITHELIAL CELLS

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The alveolar epithelium is a critical target for toxic agents, especially inhaled PM whose size profile predicts mostly alveolar deposition. Epithelial cells are not passive targets, they can synthesize and release cytokines, lipid mediators and ROS in response to a number of stimuli, thereby contributing to inflammation. We studied the role of ROS as mediators of the lung responses to Concentrated Ambient Particles (CAPs) on lung epithelial cells. Primary cultures of alveolar type II cells were exposed to CAPs, quartz or TiO_2. The intracellular concentration of H_2O_2 was measured at different time points after addition of the particles using the peroxidase-dependent oxidation of scopoletin. H_2O_2 concentrations were increased by >2-fold by CAPs suspensions (CAPs: 0.48±0.03 μM, control: 0.23±0.05 μM). Quartz particles also show strong effects on H_2O_2 concentrations (0.43±0.07 μM), whereas inert TiO_2 particles did not change H_2O_2 values (0.26±0.07 μM). Different CAPs samples collected on different days showed different effects on H_2O_2 concentrations when administered at the same final concentration. Interestingly, some of the CAPs preparations had stronger effects than the highly pathogenic α-quartz particles. Exposure of lung epithelial cells to CAP but not to TiO_2 increased their release of LDH (CAPs: 170±20 U/ml; control: 100±7 U/ml) and MIP-2 (0.23±0.02 ng/ml, control: 0.16±0.02 ng/ml) into the medium, and caused a significant increase in the number of apoptotic cells (20±5%, 34±5%). CAPs effects on LDH and MIP-2 release were completely abolished by pre-incubation with 1μM catalase, indicating that H_2O_2 is necessary for CAPs toxicity.

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SEASONALITY OF PROOXIDANT CONDITIONS IN THE MEDITERRANEAN DEMOSPONGE PETROSIA FICIFORMIS.

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Symbioses have been largely described between photosynthesizing microalgae and marine invertebrates including porifera. Photosynthetic products are utilized as additional food source in the host tissue which are also exposed to increased levels of photosynthetically produced ROS. Since Mediterranean symbioses naturally experience marked seasonal variations in the content of symbionts, light intensity and seawater temperature, the aim of this work was to investigate if these fluctuations modulate the prooxidant challenge to sponge tissues. The antioxidant efficiency of P. ficiformis was characterized on a monthly basis by integrating the analysis of the main antioxidants (superoxide dismutase, catalase, glutathione S-transferases, glutathione reductase, glutathione peroxidases) with the measurement of total oxyradical scavenging capacity (TOSC) which provided a more holistic assessment of the capability of sponge tissues to absorb different forms of reactive oxygen species (such as peroxyl radicals and hydroxyl radicals). Symbiotic sponges showed a significant enhancement of antioxidant defences and marked seasonal changes in tissues directly exposed to photosynthetically produced oxyradicals. The marked increase of catalase and TOSC in summer suggests a greater formation of H2O2 in the symbioses supporting the hypothesis that seawater temperature can significantly modulate the prooxidant challenge also in Mediterranean symbioses. These results suggest that species with lower antioxidant efficiency might be less tolerant and more susceptible to conditions of oxidative damages caused by anomalous increases of temperature during the summer months.

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ENVIRONMENTAL PROOXIDANT CONDITIONS MODULATE THE ANTIOXIDANT EFFICIENCY IN EARLY LIFE STAGES OF ANTARCTIC SILVERFISH (PLEURAGRAMMA ANTARCTICUM)

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Antarctic summer is characterized by high levels of solar irradiance and UV radiation which, during the ice melting cause the photochemical activation of dissolved organic carbon and production of hydrogen peroxide in Antarctic sea water. Organisms living in the photic zone are exposed to such prooxidant conditions and early life stages might be particularly susceptible. The basal efficiency of antioxidant system was characterized in embryos and larvae of the silverfish Pleuragramma antarcticum, a pelagic species with a pivotal role in Antarctic food-webs which has an important nursery area between platelet ice crystals occurring beneath the sea ice.

Embryos and larvae were firstly collected in early November and found at the sea-ice interface only during the following 2 weeks. During this period many antioxidant defences, and especially catalase and glutathione peroxidases, significantly increased. The total oxyradical scavenging capacity (TOSC) toward peroxyl radicals did not exhibit significant differences, while the overall capability to neutralise hydroxyl radicals tended to decrease in the second week. These variations indicate a temporal enhancement of environmental prooxidant pressure for larvae and the role of the H2O2-HO• pathway in the period anticipating sea ice melting, when elevated solar radiation reach the sea surface. Early life stages of P. antarcticum exposed to B[a]P in laboratory

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