Abstract

Background: Epidemiologic studies associate childhood exposure to traffic-related air pollution with increased respiratory infections and asthmatic and allergic symptoms. The strongest associations between traffic exposure and negative health impacts are observed in individuals with respiratory inflammation. We hypothesized that interactions between nitric oxide (NO), increased during lung inflammatory responses, and reactive oxidative species (ROS), increased as a consequence of traffic exposure — played a key role in the increased susceptibility of these at-risk populations to traffic emissions.

Methods: Diesel exhaust particles (DEP) were used as surrogates for traffic particles. Murine lung epithelial (LA-4) cells and BALB/c mice were treated with a cytokine mixture (cytomix: TNFα, IL-1β, and IFNγ) to induce a generic inflammatory state. Cells were exposed to saline or DEP ($25\mu g/cm^2$) and examined for differential effects on redox balance and cytotoxicity. Likewise, mice undergoing nose-only inhalation exposure to air or DEP ($2mg/m^3$ x 4h/d x 2d) were assessed for differential effects on lung inflammation, injury, antioxidant levels, and phagocyte ROS production.

Results: Cytomix treatment significantly increased LA-4 cell NO production though iNOS activation. Cytomix+DEP-exposed cells incurred the greatest intracellular ROS production, with commensurate cytotoxicity, as these cells were unable to maintain redox balance. By contrast, saline+DEP-exposed cells were able to mount effective antioxidant responses. DEP effects were mediated by: (1) increased ROS including superoxide anion ($O_2^{\cdot -}$), related to increased xanthine dehydrogenase expression and reduced cytosolic superoxide dismutase activity; and (2) increased peroxynitrite generation related to

interaction of $O_2^{\cdot -}$ with cytokine-induced, NO. Effects were partially *reduced* by SOD supplementation or by blocking iNOS induction. In mice, cytomix+DEP-exposure resulted in greater ROS production in lung phagocytes. Phagocyte and epithelial effects were, by and large, *prevented* by treatment with FeTMPyP, which accelerates peroxynitrite catalysis.

Conclusions: During inflammation, due to interactions of NO and $O_2^{\cdot -}$, DEP-exposure was associated with nitrosative stress in surface epithelial cells and resident lung phagocytes. As these cell types work in concert to provide protection against inhaled pathogens and allergens, dysfunction would predispose to development of respiratory infection and allergy. Results provide a mechanism by which individuals with pre-existing respiratory inflammation are *at increased risk* for exposure to traffic-dominated urban air pollution.

Keywords: Traffic, Diesel, Particles, Epithelial cells, Phagocytes, Nitric oxide, Peroxynitrite, Redox balance