Final Technical Report

Date of Final Report: August 31, 2006
EPA Grant Number: R827352C002
Center Name: Southern California Particle Center and Supersite (SCPCS)
Center Director: John R. Froines
Title: Pro-inflammatory and the Pro-oxidative Effects of Diesel Exhaust Particulate in Vivo and in Vitro
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Project Period: June 1, 1999–May 31, 2005 (no-cost extension to May 31, 2006)
Period Covered by the Report: June 1, 1999–May 31, 2006
RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Topic A: Studies Emphasizing Investigation of the Biological Mechanisms of Particulate Matter (PM) Effects in Relation to PM Physical and Chemical Characteristics

Objective(s) of the Research Project: Research in this project has increased our understanding of the mechanisms by which PM induce adverse health effects. Progress has been made in understanding the oxidative stress pathways by which diesel exhaust particulate (DEP) and ambient PM mediate injury, and has also helped to elucidate the adjuvant effects of DEP in asthma. We will address research findings and conclusions from our work according to three project aims.

Summary of Findings:

Aim 1: To Elucidate the Role of Reactive Oxygen Species (ROS) and Inflammation in PM-Induced Adverse Health Effects In Vitro and In Vivo

A potential mechanistic link between PM exposures and inflammation involves the generation of ROS and oxidative stress (Li, et al., 2003; Nel, 2005). A number of our studies, described in more detail below have demonstrated that ambient PM and DEP induce ROS production in target cells such as macrophages and bronchial epithelial cells (Li, et al., 2003a; Nel, 2005; Li, et al., 2002a; Li, et al., 2002b; Hiura, et al., 1999; Li, et al., 2003b). DEP were used in our studies as a convenient model for vehicular ultrafine particles (UF), which are a common component of ambient aerosols in urban areas.

We performed a series of in vitro and in vivo experiments to explore the link between ROS production, oxidative stress and inflammatory tissue injury (Li, et al., 2003a; Nel, 2005; Mingi, et al., 2003). The findings of the studies form the basis for the development of a hierarchical oxidative stress model (Figure 1). The model posits that at lower levels of oxidative stress (Tier 1), there is an induction of phase II enzymes regulated by a genetic response pathway that involves the transcription factor, Nrf2 (Li, et al., 2003a; Nel, 2005; Li, et al., 2002b; Gilmour, et
Nrf2 drives the antioxidant response element (ARE) in the promoter of phase II response genes, leading to the expression of antioxidant and detoxification enzymes (Li, et al., 2004). Treatment of target cells in vitro with DEP, organic DEP extracts or ambient UF induced the expression of heme oxygenase 1 (HO-1), glutathione-S-transferase (GST), NADPH quinone oxidoreductase (NQO1), catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glucuronosyltransferase (UGT) (Li, et al., 2004; Li, et al., 2000). These phase II enzymes protect against oxidative stress injury (Tiers 2 and 3), and a reduced Tier 1 defense could therefore promote PM susceptibility (Li, et al., 2003a; Nel, 2005). In humans, reduced defenses against oxidative stress can result from phase II enzyme polymorphisms, e.g., the GST M1 null genotype which predisposes to the development of asthma and enhanced sensitization to common environmental allergens during nasal DEP challenge (Li, et al., 2003a). Conversely, induction of a phase II response may be a key factor in adaptation to a polluted environment, and may explain why persistent inflammatory changes in the lung are not observed after repeated exposure to low concentrated ambient particle (CAP) levels. Some phase II enzymes, such as HO-1, exert anti-inflammatory effects based on their ability to interfere with pro-inflammatory response pathways (Li, et al., 2000; Li and Nel, 2006).

**Figure 1. Hierarchical Oxidative Stress Model**

If Tier 1 protection fails, our model proposes that further increase in oxidative stress generates pro-inflammatory responses (Tier 2) or cytotoxic effects (Tier 3) depending upon the level of insult and response capability of the exposed cells (Figure 1). Tier 2 responses are linked to the activation of intracellular signaling pathways which impact cytokine and chemokine production (Li, et al., 2003a). An example is activation of the MAP kinase cascade (Li, et al., 2003a). This cascade is responsible for the expression and activation of AP-1 transcription factors (e.g., c-Jun and C-Fos), which in turn are responsible for the expression of a variety of pro-inflammatory genes, including those encoding for cytokines, chemokines and adhesion molecules. Tier 3 responses in our model involve mitochondrial perturbation by pro-oxidative chemicals (Hiura, et al., 1999; Li, et al., 2003b; Hiura et al., 2000; Xia, et al., 2004). Although the in vivo significance of the mitochondrial pathway is uncertain, we have demonstrated in tissue culture cells that PM-induced interference in one electron transfers in the mitochondrial inner membrane and perturbation of the mitochondrial permeability transition pore (PTP) can contribute to

The principles of a hierarchical oxidative stress response were tested in macrophages and epithelial cell cultures exposed to ambient UF, DEP extracts, or fractionated DEP extracts (Li, et al., 2002a). At the lowest tier of oxidative stress (Tier 1), the expression of catalase, SOD, and HO-1 indicates the involvement of Nrf2-regulated enzymes that can suppress inflammation through their antioxidant activities (Li, et al., 2003a; Li, et al., 2004). This finding was extended by showing that particulate pollutants increase the accumulation of Nrf2 in the nucleus and activate the ARE (Li, et al., 2004). Interestingly, the buildup of Nrf2 in the nucleus is dependent on a prolongation of protein half-life by interference in proteosomal degradation (Li, et al., 2004). Activation of the ERK, p38 and Jun kinase cascades was confirmed by phosphor-proteome analysis (Wang, et al., 2005). To further substantiate the findings, related experiments are now being conducted in vivo, using BAL fluid and lung tissue from PM-exposed animals to find in vivo biomarkers of oxidative stress. These markers could be useful to identify the subsets of the human population susceptible to PM exposure.

While there is still considerable debate about which particle components are responsible for the pro-oxidative and pro-inflammatory effects associated with PM, our work adds to accumulating evidence that transition metals, such as copper, vanadium, chromium, nickel, cobalt and iron, as well as aromatic and polar organic substances play a role in ROS production (Li, et al., 2003a; Li, et al., 2000). The particle backbone could play an important role in acting as a template for single electron transfers reactions, including electron transfer to molecular dioxygen (Figure 2). This could involve redox cycling reactions, as demonstrated by the ability of ambient PM samples to generate superoxide in the presence of dithiothreitol (DTT) (Li, et al., 2003b). DTT oxidation can be assessed by a colorimetric reaction to assay for the content of redox cycling chemicals in urban PM samples (Li, et al., 2003b). In addition, biologically catalyzed oxidation-reduction reactions in the cellular interior, as well as interference in one electron transfers in the mitochondrial inner membrane, contribute to ROS generation (Xia, et al., 2004). In addition to the ability of ROS to damage cellular proteins, DNA and cell membranes, electrophilic PM chemicals such as the quinones can modify cellular proteins by Michael acceptor reactions (Li, et al., 2004). It is likely that this type of reaction leads to Nrf2 release to the nucleus by the covalent modification of its cytosolic chaperone, Keap-I (Li, et al., 2004). The covalent modification of intracellular and tissue proteins was also confirmed by studying their tyrosylation and carbonylation after in vivo exposure to diesel exhaust particulate (Whitekus, et al., 2002).
In experiments to characterize the redox cycling chemicals present in PM, silica gel chromatography was used to fractionate organic DEP extracts (Li, et al., 2002a; Li, et al., 2003b; Li, et al., 2000; Xia, et al., 2004). Aliphatic, aromatic and polar chemical fractions were eluted by increasingly polar solvents and tested for reactivity in the DTT assay. The quinone-enriched polar material was more active than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction. Glutathione depletion in epithelial cells and macrophages is associated with the exposure to DEP extracts and with activity in the DTT assay (Li, et al., 2002a; Li, et al., 2003b; Li, et al., 2000; Xia, et al., 2004). The aliphatic fraction was inactive in these assays.

The relationship between the organic chemical composition and the redox cycling potential of PM that had been noted for diesel particles was confirmed in a study in which UF were compared to coarse particles (C) and fine particles (F) collected in the Los Angeles Basin (LAB) (Li, et al., 2003b). UF were more active than C and F in the DTT assay, and were also more prone to generate oxidative stress in macrophages and epithelial cells (Li, et al., 2003b). Both the in vitro and cellular responses showed an excellent correlation with the PAH content of UF (Nel, 2005; Li, et al., 2003b). Another important observation in this study was the ability of UF to lodge in and disrupt the mitochondrial architecture (Li, et al., 2003b). This finding is related to cellular apoptosis and apo-necrosis by a pathway that requires opening of the mitochondrial PTP (Hiura, et al., 2000; Xia, et al., 2004). Functional effects on the PTP and inability to sustain one electron transductions in the mitochondrial inner membrane was confirmed in isolated mitochondrial preparations through the use of calcium-dependent swelling, calcium retention capacity and dissipation of the mitochondrial membrane potential (Xia, et al., 2004). Moreover, UF particle effects could be reproduced by polar and aromatic chemicals fractionated from DEP, while commercial polystyrene nanoparticles were inactive (Xia, et al., 2004). These data demonstrate differential particle toxicity associated with particle size, composition, and subcellular localization.

Aim 2: To Develop a Murine Model for Asthma to Explain the Adjuvant Effects of DEP on Ovalbumin (OVA)-Induced Allergic Inflammation and Airway Hyperreactivity (AHR)

The asthma studies were premised on findings that DEP enhance allergen-specific IgE and TH2 cytokine production in humans and animals (Li, et al., 2003a; Nel, 2005). We demonstrated that aerosolized DEP can enhance OVA-specific IgE production in a murine inhalation model (Whitekus, et al., 2002). The adjuvant effect of DEP could be suppressed by NAC administration (Whitekus, et al., 2002). While adequate for upregulating IgE production, an
Aim 3: Dosimetry and Distribution of Particles

Health effects of exposure to PM are likely to be proportional to the PM dose to critical cells and organs. Tissue dose is influenced by the proportion of inhaled particles that are retained in the lung with each breath. The proposed hierarchical oxidative stress response that occurs in PM target cells has been discussed above. A frequently asked question is how the experimental in vitro DEP concentrations that span the three tiers of oxidative stress (e.g., 1–100 µg/ml in macrophages) can be understood in terms of tissue concentrations that are achieved in the lung during real-life exposures. One method to reconcile doses used in vitro with in vivo exposures, is to convert ambient PM levels, measured in µg/m³, to a dose that is deposited on a planar surface and then to compare that to the calculated dose of the DEP that are deposited on a planar tissue culture surface (Li, et al., 2003a). Our calculations resulted in a planar concentration of 0.2–20 µg/cm² on the tissue culture dish. This concentration was compared to a theoretical in vivo deposition dose that would occur in the nasopharyngeal (NPR), tracheobronchial (TBR), and alveolar (AVR) regions of the respiratory tract of an adult person exposed to PM₂₅ in Rubidoux, California (Li, et al., 2003a). After correction for parameters such as airway anatomy,
nasal breathing, high rates of deposition at bifurcation points, and uneven airflow due to airway obstruction in asthma or chronic obstructive pulmonary disease (COPD), the calculated deposition values for the NPR, TBR and AVR were 204, 2.3 and 0.05 µg/cm², respectively, for the Rubidoux scenario. The calculations showed that it is possible to achieve doses in the nose and TBR from ambient exposures that are responsible for the in vitro induction of antioxidant, pro-inflammatory and cytotoxic responses.

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**Supplemental Keywords:** NA

**Relevant Web Sites:** http://www.scpcs.ucla.edu