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Interactive effects of cerium oxide and diesel exhaust nanoparticles on inducing pulmonary fibrosis

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ABSTRACT

Cerium compounds have been used as a fuel-borne catalyst to lower the generation of diesel exhaust particles (DEPs), but are emitted as cerium oxide nanoparticles (CeO_2) along with DEP in the diesel exhaust. The present study investigates the effects of the combined exposure to DEP and CeO_2 on the pulmonary system in a rat model. Specific pathogen-free male Sprague–Dawley rats were exposed to CeO_2 and/or DEP via a single intratracheal instillation and were sacrificed at various time points post-exposure. This investigation demonstrated that CeO_2 induces a sustained inflammatory response, whereas DEP elicits a switch of the pulmonary immune response from Th1 to Th2. Both CeO_2 and DEP activated AM and lymphocyte secretion of the proinflammatory cytokines IL-12 and IFN-γ, respectively. However, only DEP enhanced the anti-inflammatory cytokine IL-10 production in response to ex vivo LPS or Concanavalin A challenge that was not affected by the presence of CeO_2, suggesting that DEP suppresses host defense capability by inducing the Th2 immunity. The micrographs of lymph nodes show that the particle clumps in DEP + CeO_2 were significantly larger than CeO_2 or DEP, exhibiting dense clumps continuous throughout the lymph nodes. Morphometric analysis demonstrates that the localization of collagen in the lung tissue after DEP + CeO_2 reflects the combination of DEP-exposure plus CeO_2-exposure. At 4 weeks post-exposure, the histological features demonstrated that CeO_2 induced lung phospholipidosis and fibrosis. DEP induced lung granulomas that were not significantly affected by the presence of CeO_2 in the combined exposure. Using CeO_2 as diesel fuel catalyst may cause health concerns.

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Introduction

Cerium, a member of the lanthanide series of metals, is the most abundant of the rare-earth elements in the Earth's crust (average concentration of 50 ppm) (Hedrick, 2004). Cerium oxide has been used commercially in polishing agents, television tubes, and precision optics and it is also applied in various consumer products including semiconductors (EPA, 2009). Recently cerium oxide has been used as a fuel borne catalyst in combination with a particulate filter in diesel engines to enhance combustion by reducing the ignition temperature of the carbonaceous particulate on the filter; thus, improving fuel burning efficiency and substantially decreasing particle mass in the exhaust. Recent studies demonstrated that cerium was generated in the diesel exhaust from an engine using standard diesel fuel spiked with either cerium oxide or suspension of "Envirox" (Cassee et al., 2012).

Diesel exhaust particles (DEPs) are carbon-based particles containing various organic compounds, including polycyclic aromatic hydrocarbons and nitroaromatic compounds adsorbed onto the carbonaceous core (Schuetzle, 1983; Schuetzle et al., 1981). Diesel exhaust is a complex and variable mixture of gases, vapors, and particulates containing numerous chemicals. Usage of diesel engines by various industries is increasing because of fuel efficiency. However, diesel engines emit 30–100 times more particulate matter (PM) than gasoline engines. The environmental health concerns for DEP stem from their substantial levels in urban and industrial areas as a major component of airborne PM, and the fact that epidemiological studies have demonstrated an association between exposures to PM and increased respiratory mortality and morbidity (Dockery et al., 1993).

Abbreviations: AM, alveolar macrophage; Arg-1, arginase-1; BAL, bronchial alveolar lavage; CeO_2, cerium oxide; ConA, Concanavalin A; DEP, diesel exhaust particle; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; IPF, idiopathic pulmonary fibrosis; MMP, matrix metalloproteinase; OPN, osteopontin; PM, particulate matter; PL, phospholipids; RE, rare earth; ROS, reactive oxygen species; TGF, transforming growth factor; TIMP, tissue inhibitors of matrix metalloproteinase; TEM, transmission electron microscopy.

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Animal studies have shown that both the organic and the particulate components of DEP cause oxidant lung injury but trigger different cellular responses (Ma and Ma, 2002). Long-term exposure to DEP has been shown to induce tumor formation in rodents (Mauderly et al., 1987). Acute exposure to DEP induces pulmonary inflammation, activates alveolar macrophages (AMs), and alters the pulmonary immune/inflammatory responses to environmental allergens and bacterial infections (Dong et al., 2005; Yang et al., 1999, 2001; Yin et al., 2004). DEP is known to induce a change in pulmonary immune response that weakens the innate and cell-mediated immunity while enhances adaptive immune responses (Dong et al., 2005; Yin et al., 2004). Studies have shown that Th2 cytokines promote the differentiation of profibrogenic macrophages and the development of fibrotic diseases (Wynn, 2004).

With the addition of cerium in diesel fuel, the potential health effects associated with exposures to cerium oxide alone and in combination with DEP are not yet clear. This is consistent with the general consensus as to the high degree of uncertainty related to the environmental and health implications of manufactured-engineered nanomaterials (Cassee et al., 2011; Park et al., 2007; EPA, 2009). Cerium is known to induce rare earth pneumoconiosis characterized by accumulation of cerium particles (and other rare earth particles) in the lungs and lymphoreticular system after prolonged occupational exposure to cerium fumes or dust (Pairon et al., 1994, 1995; Porru et al., 2001; Sabbioni et al., 1982; Sulotto et al., 1986). Exposure was not quantified in any of these cases. The pathologic features of this rare earth pneumoconiosis include interstitial fibrosis, granulomas, and bilateral nodular chest lesions. A common feature in this disease is the accumulation of cerium particles in the alveoli and interstitial tissue that persists even decades after exposure was ended (Pairon et al., 1994).

A previous study carried out in our laboratory demonstrated that exposure of rats to cerium oxide nanoparticles (CeO_2) by a single intratracheal instillation induced a sustained dose dependent pulmonary inflammatory response through 4 weeks post-exposure (Ma et al., 2011). At the end of 4 weeks, AM was transformed from the classic activated, inflammatory subset of M1 to the alternatively activated or fibrogenic subset M2, with a significant increase in arginase-1 expression (Ma et al., 2011). Pulmonary fibrosis was evident in the CeO_2-exposed lungs at 28 days post-exposure and the presence of CeO_2 in the lung tissue was demonstrated (Ma et al., 2011; Ma et al., 2012). These studies have also shown that the CeO_2 exposure not only increased production of the fibrotic cytokine, transforming growth factor (TGF)-β1 and osteopontin (OPN), by AM, but also induced a range of mediators such as matrix metalloproteinases (MMPs), i.e., proteolytic enzymes involved in the degradation of extracellular matrix (ECM) collagens, and tissue inhibitor for MMP (TIMP), involved in the lung tissue remodeling. The imbalance of MMP-9/TIMP-1 may play an important role in the development of fibrosis.

Both CeO_2 and DEP caused severe lung injury. However, they exhibited different effects on pulmonary cellular responses. DEP exposure induced acute pulmonary inflammation that recovered with time, but a strong effect on lymphocyte differentiation that significantly suppressed the pulmonary self-defense capability against bacterial infection (Chan et al., 1981; Yin et al., 2002, 2003). In contrast, CeO_2 induced sustained inflammatory responses, which led to lung fibrosis. The presence of CeO_2 in diesel exhaust emissions, thus, may represent a serious occupational and environmental health risk with pulmonary fibrosis as a plausible end point. The objective of the current study is to characterize the effects of the presence of CeO_2 in DEP on pulmonary responses, including modification of DEP-induced cellular responses and lung fibrosis. Specifically, the present study investigates the combination exposure of DEP plus CeO_2 on lung inflammation and injury; lymphocyte responses and pulmonary defense capability; and development of fibrotic lung lesions.

Materials and methods

Materials

Specific pathogen-free male Sprague–Dawley (Hla:SD-CVF) rats (6 weeks old, ~200 g) were purchased from Hilltop Laboratories (Scottdale, PA). Rats were kept in cages individually ventilated with HEPA-filtered air, housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved facility and provided food and water ad libitum. A standardized DEP sample (standard reference material 2975) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD). Cerium oxide nanoparticles, 10 wt.% in water, were obtained from Sigma-Aldrich (St Louis, MO, USA).

Particle preparation

To prepare particle suspension, DEP, CeO_2 or DEP + CeO_2 was suspended in sterile saline then sonicated for 1 min using an ultrasonic processor (Heat System-Ultrasonics, Plainview, NY, USA). Particle suspension was prepared immediately before usage and was vigorously vortexed to provide well mixed suspension immediately before each instillation, it occurred less than 1 min later. In this practice, we did not experience the separation of the particles in the suspension. However, if one lets the suspension stand alone for some time the particles would separate out in the suspension. That was the reason for vortexing of the sonicated suspension.

Animal exposures

All rats were exposed and sacrificed according to a standardized experimental protocol that complied with the Guide for the Care and Use of Laboratory Animals and was approved by the National Institute for Occupational Safety and Health Animal Care and Use Committee. Animals were used after a 1 week acclimatization period. For particle exposure, rats were anesthetized with sodium methohexital (35 mg/kg, i.p.) and placed on an inclined restraint board. Rats were exposed to 0.3 ml suspensions of cerium oxide at a final concentration of 0.15, 0.5, 1, 3.5 or 7 mg/kg body weight, DEP (35 mg/kg), or CeO_2 + DEP via a single intratracheal instillation. Saline (0.9% NaCl) was administered to control rats. The treated animals (at least six in each treatment group) were sacrificed at 1, 3, 10 or 28 days post-exposure.

Particle characterization

The primary particle size and size of the particles as instilled have been characterized previously. The diameter of the primary CeO_2 particle is in the range of 6.4–14.8 nm with a mean of 9.26 ± 0.58 nm, determined by field emission scanning electron microscopy (FESEM). The diameter of primary particle was also determined to be in the range of 6.25–17.5 nm with a mean diameter of 10.14 ± 0.76 nm using transmission electron microscopy (TEM) (Nalabotu et al., 2011). We have also reported previously dynamic light scattering (DLS) of nanoparticles as diluted in saline for intratraceal instillation. These particles agglomerate in saline, with DLS showing a major particle peak at 2.5 μm and a small subpopulation with average size of 0.3 μm (Ma et al., 2011). The surface area of the particles used is in the range of 80–100 m^2/g using BET (Sigma Chemicals). The purity of the CeO_2 samples used in this study has been determined previously (Park et al., 2007; Yokel et al., 2009). The sum of the contamination from lead, aluminum, copper, titanium, iron, nickel and zinc was <0.2% of the Ce concentration according to ICP-MS analysis.