Toxicology and Applied Pharmacology 288 (2015) 63–73

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

# Effects of amorphous silica coating on cerium oxide nanoparticles induced pulmonary responses

Jane Ma [a,*], Robert R. Mercer [a], Mark Barger [a], Diane Schwegler-Berry [a], Joel M. Cohen [b], Philip Demokritou [b], Vincent Castranova [c]

[a] Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA
[b] Harvard TH Chan School of Public Health, Harvard University, Boston, MA, USA
[c] School of Pharmacy, West Virginia University, Morgantown, WV, USA

ARTICLE INFO

*Article history:*
Received 21 May 2015
Revised 14 July 2015
Accepted 16 July 2015
Available online 22 July 2015

*Keywords:*
Cerium oxide, amorphous silica
Lung inflammation
Pulmonary fibrosis
Safer by design

ABSTRACT

Recently cerium compounds have been used in a variety of consumer products, including diesel fuel additives, to increase fuel combustion efficiency and decrease diesel soot emissions. However, cerium oxide ($CeO_2$) nanoparticles have been detected in the exhaust, which raises a health concern. Previous studies have shown that exposure of rats to nanoscale $CeO_2$ by intratracheal instillation (IT) induces sustained pulmonary inflammation and fibrosis. In the present study, male Sprague–Dawley rats were exposed to $CeO_2$ or $CeO_2$ coated with a nano layer of amorphous $SiO_2$ ($aSiO_2/CeO_2$) by a single IT and sacrificed at various times post-exposure to assess potential protective effects of the $aSiO_2$ coating. The first acellular bronchoalveolar lavage (BAL) fluid and BAL cells were collected and analyzed from all exposed animals. At the low dose (0.15 mg/kg), $CeO_2$ but not $aSiO_2/CeO_2$ exposure induced inflammation. However, at the higher doses, both particles induced a dose-related inflammation, cytotoxicity, inflammatory cytokines, matrix metalloproteinase (MMP)-9, and tissue inhibitor of MMP at 1 day post-exposure. Morphological analysis of lung showed an increased inflammation, surfactant and collagen fibers after $CeO_2$ (high dose at 3.5 mg/kg) treatment at 28 days post-exposure. $aSiO_2$ coating significantly reduced $CeO_2$-induced inflammatory responses in the airspace and appeared to attenuate phospholipidosis and fibrosis. Energy dispersive X-ray spectroscopy analysis showed Ce and phosphorous (P) in all particle-exposed lungs, whereas Si was only detected in $aSiO_2/CeO_2$-exposed lungs up to 3 days after exposure, suggesting that $aSiO_2$ dissolved off the $CeO_2$ core, and some of the $CeO_2$ was transformed to $CePO_4$ with time. These results demonstrate that $aSiO_2$ coating reduce $CeO_2$-induced inflammation, phospholipidosis and fibrosis.

Published by Elsevier Inc.

## 1. Introduction

Cerium, a lanthanide member of the rare earth (RE) metals, has a variety of industrial applications and recently has been used as diesel fuel additive in conjunction with a particulate filter to reduce the ignition temperature of the carbonaceous diesel exhaust particles (DEPs). This results in more efficient burning of DEP and the regeneration of the particulate filter (HEI, 2001; Prospect, 2009). Using cerium as a catalyst substantially decreases both particle mass (>90%) and number (99%) in the diesel exhaust; however, a small amount of cerium oxide ($CeO_2$) nanoparticles is emitted in the particulate phase of the exhaust (HEI, 2001). Other studies further demonstrated that cerium was generated in the diesel exhaust from an engine using standard diesel fuel spiked with either $CeO_2$ or suspension of "Envirox", a commercial diesel fuel combustion catalyst based on $CeO_2$ (Cassee et al., 2012).

Animal studies have demonstrated that exposure of rats to nano scale $CeO_2$ by intratracheal instillation induced persistent lung inflammation and injury throughout a 28 day post-exposure period with the retention of particles in the exposed lungs (Ma et al., 2011; Molina et al., 2014). Other studies have shown that intratracheal exposure of $CeO_2$ induced pulmonary inflammation and small granulomas in both rats (Toya et al., 2010) and mice (Park et al., 2010), and that exposure of mice to $CeO_2$ through head and nose inhalation caused chronic inflammatory responses (Srinivas et al., 2011). Our previous studies have demonstrated that $CeO_2$ exposure induced pulmonary phospholipidosis, activated alveolar macrophage (AM) production of inflammatory cytokines, and induced fibrogenic and extracellular membrane (ECM) mediator production leading to pulmonary fibrosis (Ma et al., 2012).

Pulmonary fibrosis is characterized by an excessive deposition of extracellular matrix in the interstitium, where fibroblasts play a major role in the reconstruction of damaged connective tissue by producing new ECM components. The balance between ECM synthesis and degradation of matrix components is crucial for tissue repair, a process that requires a balance between matrix metalloproteinases (MMPs), which represent

* Corresponding author at: PPRB/HELD, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505-2888, USA.
*E-mail address:* jym1@cdc.gov (J. Ma).

http://dx.doi.org/10.1016/j.taap.2015.07.012
0041-008X/Published by Elsevier Inc.

a family of extracellular and cell surface-associated proteinases, and tissue inhibitors of matrix metalloproteinases (TIMPs). Abnormal activation of proteolytic and/or antiproteolytic functions can lead to lung diseases, including fibrosis (Gueders et al., 2006). Indeed, in human idiopathic pulmonary fibrosis (IPF), ECM accumulation with upregulated fibroblast proliferation has been demonstrated to result from excessively elevated TIMPs compared to MMPs, leading to a non-degrading fibrillar collagen microenvironment (Selman et al., 2000).

Diesel exhaust exposure alone induces adverse cardiopulmonary effects. The use of cerium as fuel catalyst leads to altered emission characteristics and induced more adverse pulmonary effects than DEP (Ma et al., 2014; Snow et al., 2014). The suggested effects of the combination of DEP and $CeO_2$, thus, raises concerns of health effects due to the presence of $CeO_2$ in diesel exhaust. The translocation of $CeO_2$ from the lung to other organs via circulation has also been demonstrated in the $CeO_2$-exposed animals (He et al., 2010; Ma et al., 2014; Molina et al., 2014; Nalabotu et al., 2011). These studies demonstrate that nano-ceria could penetrate through the alveolar wall into the systemic circulation and accumulate in the extrapulmonary organs, including lymph nodes and liver, leading to more adverse health concerns when using cerium as a diesel fuel catalyst.

It has been reported in a rat model that nearly 80% of the instilled $CeO_2$ was deposited in the lung at 24 h post-instillation. In addition, ~64% of the given $CeO_2$ remained in the lung 28 days after exposure, with an elimination half-life of 103 days (He et al., 2010). In vitro studies revealed minimal translocation (<0.1%) of $CeO_2$ across alveolar epithelial monolayers via trans- and para-cellular pathways (Cohen et al., 2014). Tissue distribution studies demonstrated that $CeO_2$ particles were detected in AM, mixed with accumulated lung surfactant in the alveolar air space, and in the alveolar interstitial tissue at 28 days post-exposure (Ma et al., 2011). Semmler-Behnke et al. (2007) proposed a long-term nanoparticle clearance mechanism, involving AM-mediated translocation of particles to the interstitial lymphatics and removal towards the larynx with subsequent re-entrainment into the airway lumen. Considering the long elimination half-life and the ceria/cerium-related pathogenesis of pneumoconiosis (McDonald et al., 1995; Porru et al., 2001; Sabbioni et al., 1982), exposure to the increased $CeO_2$ in diesel exhaust and from $CeO_2$ enabled products has raised health concerns. Recently, a "safer by design" concept for reducing the $CeO_2$-mediated toxicity has been pursued. A concept is based on encapsulation of flame generated nanoparticles with a nanothin amorphous silica ($aSiO_2$) layer during their synthesis in aerosol reactors, as reported by Gass et al. (2013). Based on this concept, nanoparticles of $CeO_2$ and $aSiO_2$-nanothin coated $CeO_2$ ($aSiO_2/CeO_2$) aerosols were generated by the Harvard Versatile Engineered Nanomaterial Generating System (VENGES) directly connected to a whole-body animal inhalation chamber, and the pulmonary inflammatory responses were monitored (Demokritou et al., 2012). The most important feature of VENGES is that the freshly generated particles of nano size directly entered the animal exposure chamber, while the commonly used aerosol generators, which disperse nanopowders, produce aged, agglomerated particles (Fischer and Chan, 2007; Schmoll et al., 2009). Based on this design, our studies have demonstrated that coating of $CeO_2$ with $aSiO_2$ substantially reduced $CeO_2$-induced acute lung inflammation and cytotoxicity at 24 h post-exposure (Demokritou et al., 2012).

The objective of the present study is to investigate further pulmonary responses to $aSiO_2$-coated $CeO_2$ ($aSiO_2/CeO_2$), $CeO_2$ and $aSiO_2$ nanoparticles in a time- and dose-dependent manner. The stability of $aSiO_2$ coating on $CeO_2$, and the development of pathological changes in $CeO_2$-, $aSiO_2/CeO_2$- and $aSiO_2$-exposed lungs were also evaluated.

## 2. Methods

### 2.1. Animals

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 Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC)-approved facility, and provided food and water ad libitum. Animals were used after a 1 week acclimation period. All rats were exposed and euthanized according to a standardized experimental protocol that complied with the Guide for the Care and Use of Laboratory Animals and was approved by the institutional Animal Care and Use Committee.

### 2.2. Particle generation and characterization

$CeO_2$, $aSiO_2/CeO_2$ and $aSiO_2$ particles were generated using the VENGES and characterized as previously described (Demokritou et al., 2012). Briefly, X-ray diffraction (XRD) patterns were obtained using a Scintag XDS2000 powder diffractometer [Cu Kα ($\lambda = 0.154$ nm), −40 kV, 40 mA, stepsize = 0.02°]. The crystal size was determined by applying the Sherrer Shape Equation to the Gaussian fit of the major diffraction peak. The Brunauer–Emmett–Teller (BET) powder-specific surface area (SSA) of all samples was measured by nitrogen adsorption at 77 K (Micromeritics TriStar; Norcross, GA), after sample degassing for 1 h at 150 °C in nitrogen. BET equivalent primary particle size was calculated, under a spherical particle assumption, using $d_{BET} = 6000/(\rho \times SSA)$, where ρ is the material density. The zeta potential (ζ) and aggregate size of the particles dispersed in water ($d_H$) were measured using dynamic light scattering (DLS) with a Malvern Zetasizer Nano-ZS instrument (Malvern Instruments Ltd., Worcestershire, UK).

### 2.3. Exposure of animals

To prepare particle suspensions, $CeO_2$, $aSiO_2/CeO_2$, or $aSiO_2$ nanoparticles were suspended in sterile water (Mediatech, Inc.; Manassas, VA) and then sonicated for 1 min using an ultrasonic processor (Heat System-Ultrasonics; Plainview, NY). Particle suspensions were prepared immediately before usage and were vigorously vortexed to provide a well-mixed suspension immediately before each instillation, which occurred less than 1 min later.

For particle exposure, rats were anesthetized with sodium methohexital (35 mg/kg, i.p.) and placed on an inclined restraint board. At final concentrations of 0.15, 1 or 3.5 mg/kg body weight, which were defined as low, medium or high concentration in this paper. There were significant differences in the density of particles used in this study. The densities for $CeO_2$, $aSiO_2/CeO_2$ and $aSiO_2$ were 7.65, 5 and 2.65 g/cm$^3$, respectively. In consideration of the different densities of the three particles, exposure doses of $aSiO_2/CeO_2$ and $aSiO_2$ were adjusted by density to the same particle number as for 0.15, 1 or 3.5 mg/kg body weight of $CeO_2$. Sterilized water was used to make nanoparticle suspensions and used as vehicle controls. The treated animals (at least six in each treatment group) were sacrificed at various time points post-exposure. The study design included different groups for the following sets of end points: inflammatory and acellular mediators; stability of $aSiO_2$ coating on the $CeO_2$ core; chemical analysis of particles in the digested lung tissues; and histological analysis of the lung tissues.

### 2.4. Isolation of AM and bronchoalveolar lavage fluid

Animals were injected with a lethal dose of euthanasia solution (sodium pentobarbital, 0.2 g/kg, i.p.) and exsanguinated by transecting the renal artery. AM were obtained by bronchoalveolar lavage (BAL) with a $Ca^{2+}$, $Mg^{2+}$-free phosphate-buffered medium (145 mM NaCl, 5 mM KCl, 1.9 mM $NaH_2PO_4$, 9.35 mM $Na_2HPO_4$, and 5.5 mM glucose; pH 7.4) as described previously (Yang et al., 2001). Briefly, the lungs were lavaged with 6 ml $Ca^{2+}$, $Mg^{2+}$-free phosphate-buffered medium in and out twice for the first lavage, and subsequently lavaged with 8 ml of the medium when ~80 ml BAL fluid (BALF) was collected from each rat. The acellular supernate from the first lavage was saved

Download English Version:

https://daneshyari.com/en/article/2568302

Download Persian Version:

https://daneshyari.com/article/2568302

Daneshyari.com