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Rat brain pro-oxidant effects of peripherally administered 5 nm ceria 30 days after exposure

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ABSTRACT

The objective of this study was to determine the residual pro-or anti-oxidant effects in rat brain 30 days after systemic administration of a 5 nm citrate-stabilized ceria dispersion. A ~4% aqueous ceria dispersion was iv-infused (0 or 85 mg/kg) into rats which were terminated 30 days later. Ceria concentration, localization, and chemical speciation in the brain was assessed by inductively coupled plasma mass spectrometry (ICP-MS), light and electron microscopy (EM), and electron energy loss spectroscopy (EELS), respectively. Pro- or anti-oxidant effects were evaluated by measuring levels of protein carbonyls (PC), 3-nitrotyrosine (3NT), and protein-bound-4-hydroxy-2-trans-nonenal (HNE) in the hippocampus, cortex, and cerebellum. Glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase levels and activity were measured in addition to levels of inducible nitric oxide (iNOS), and heat shock protein-70 (Hsp70). The blood brain barrier (BBB) was visibly intact and no ceria was seen in the brain cells. Ceria elevated PC and Hsp70 levels in hippocampus and cerebellum, while 3NT and iNOS levels were elevated in the cortex. Whereas glutathione peroxidase and catalase activity were decreased in the hippocampus, GR levels were decreased in the cortex, and GPx and catalase levels were decreased in the cerebellum. The GSH:GSSG ratio, an index of cellular redox status, was decreased in the hippocampus and cerebellum. The results are in accordance with the observation that this nanoscale material remains in this mammal model up to 30 days after its administration and the hypothesis that it exerts pro-oxidant effects on the brain without crossing the BBB. These results have important implications on the potential use of ceria ENM as therapeutic agents.

Abbreviations: 3NT, protein bound 3-nitrotyrosine; Ce, cerium; Cat, catalase; EELS, electron energy loss spectroscopy; ENM, engineered nanomaterial; GR, glutathione reductase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; Hsp70, heat shock protein 70; HNE, protein-bound 4-hydroxy-2-trans-nonenal; ICP-MS, inductively coupled plasma mass spectrometry; iNOS, inducible nitric oxide synthase; MDL, method detection limit; PC, protein carbonyl; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase; TEM, transmission electron microscopy.

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1. Introduction

Engineered nanomaterials (ENM) can be manufactured in a variety of shapes and sizes and physico-chemical, surface, as well as optical and magnetic properties. ENMs have numerous applications in research, medicine, electronics and other industries. Physico-chemical properties of nanomaterials differ from their bulk forms mainly because of the larger surface area to mass ratio, which affects reactivity, strength and electrical properties of nanomaterials. Because of their comparable size with biological molecules like proteins and DNA, ENMs can gain access to usually difficult to reach biological compartments in cells (Fubini et al., 2010). Increased surface activity can facilitate interactions with biological molecules, which may evoke greater physiological responses, different from the same basic material with larger particle size, the bulk form equivalent of ENMs (Donaldson et al., 2004; Landsiedel et al., 2009; Xia et al., 2009). One effect exhibited by ENMs is the generation of free radicals or induction of oxidative stress, which is also a primary mechanism of ENM toxicity (Xia et al., 2009). Oxidative stress effects are direct consequences of imbalance in the rates of reactive oxygen and/or nitrogen species (ROS or RNS) production verses scavenging of ROS and/or RNS and/or antioxidant levels (Butterfield et al., 2007).

Ceria ENM (a.k.a. cerium oxide; CeO_2), which is one of the most used ENM employed in different industrial applications (Yokel et al., 2009a,b; Hardas et al., 2010) has been shown to have both anti-inflammatory properties as well as potent toxicity. However, there is no clear understanding of what exactly controls ceria's pro- or anti-oxidant effects. A recently published report summarizes findings of in vitro and in vivo studies conducted with ceria ENM under basal and induced oxidative stress conditions (Celardo et al., 2011). Ceria exhibited antioxidant properties evidenced by scavenging free radicals, by reducing levels of peroxides, iNOS, TNF-α, NF-$\kappa\beta$, and interleukin, by promoting cell viability or protecting organelles from diesel exhaust and cigarette smoke-induced oxidative stress, ROS generating chemical agents, or side effects of radiation treatment. Ceria has been suggested for potential use in the treatment of diabetic cardiomyopathy, cancer, stroke, retinal degradation and Alzheimer's disease as well as to prolong life span (Chen et al., 2006; Rzigalinski et al., 2006; Das et al., 2007; Xia et al., 2008; D'Angelo et al., 2009; Hirst et al., 2009; Babu et al., 2010; Colon et al., 2010; Younce et al., 2010; Estevez et al., 2011; Niu et al., 2011). Antioxidant properties of ceria may be related to its SOD- and catalase-mimicking activity (Korsvik et al., 2007; Pirmohamed et al., 2010) attributed to Ce^{3+}/Ce^{4+} redox coupling (Celardo et al., 2011). In contrast, there are reports of ceria induced pro-oxidant effects under basal conditions. In different cell culture studies, ceria ENM mediated ROS injury, induced lipid peroxidation, caused membrane damage, led to elevation of the cytokine, IL-8, led to depletion of GSH, and led to reduced cell viability (Brunner et al., 2006; Lin et al., 2006; Park et al., 2008; Auffan et al., 2009).

To utilize ceria for therapeutic and non-therapeutic applications, it is important to know the long term effects of intended and un-intended ceria exposure on mammals. Most reports on effects of ceria ENM were conducted using non-mammalian organisms or in cell culture, and none of these addressed long-term effects or fate of ceria. In addition to our own previous studies (Yokel et al., 2009a,b; Hardas et al., 2010), a few ceria ENM studies were conducted in intact animals (Chen et al., 2006; Niu et al., 2007; Hirst et al., 2009, 2011; Amin et al., 2011; Choi et al., 2011; Srinivas et al., 2011; Zhou et al., 2011). One study reports that deposition and retention of ceria in various vital organs and increased WBC count were seen 30 days after intraperitoneal and intravenous injection to mice, but otherwise ceria was tolerated by animals (Hirst et al., 2011). Ceria reduced myocardial oxidative stress in transgenic mice for ischemic cardiomyopathy, rat liver from monocrotaline-induced ROS injury by induction of GSH levels and intravitreal injections of ceria inhibited retinal neovascular lesions (Niu et al., 2007; Amin et al., 2011; Zhou et al., 2011). However, after pulmonary inhalation of ceria ENM, granulomatous pathology and GSH depletion were seen in rat lungs (Cho et al., 2010; Srinivas et al., 2011). Animal studies have also reported that ceria ENM can accumulate in various organs, including the heart and lung, irrespective of the point of entry or distance (from injection point and organ specifically examined) when supplied as intravenous or intra-peritoneal injections or as a food additive (Chen et al., 2006; Niu et al., 2007; Hirst et al., 2009). This accumulation may lead to systemic effects involving the inflammatory response (Celardo et al., 2011) or increased ROS production under normal physiological conditions (Hirst et al., 2011).

To our knowledge there is no prior information available on the long-term effects (30 days or more after administration) of ceria on brain and how these effects may contrast with an immediate response after the initial ENM contact. Our previous study showed moderate pro-oxidant effects on rat brain, 1 and 20 h after a single acute systemic instillation of 5 nm ceria ENM (Hardas et al., 2010). The current study discusses residual effects of oxidative stress parameters in brain 30 days after one single acute ENM peripheral administration using 5 nm ceria ENM. To address the objective, the levels and activities of the antioxidant enzymes catalase, manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPx), and glutathione reductase (GR), were measured along with the ratio of reduced glutathione (GSH) to its oxidized form (GSSG). To understand the extent of changes in cellular redox status, the levels of oxidative stress endpoints, protein carbonyl (PC), 3-nitrotyrosine (3NT), and protein bound 4-hydroxyl-2-trans nonenal (HNE), were measured along with heat shock protein (Hsp70) levels.

2. Materials and methods

All the materials, methods including the well characterized 5 nm ceria ENM are same as that used in our recently published study (Hardas et al., 2010). The rats used are of the same strain, sex and approximately same weight as that used in the previous study with 5 nm ceria ENM (Hardas et al., 2010). Therefore, only a brief overview is presented.

2.1. Nanomaterial

Cerium chloride heptahydrate (Sigma–Aldrich # 228931, 99.9% metal basis), ammonium hydroxide (Fisher #3256, ACS, 28–30%) and citric acid monohydrate (EMD Chemicals Inc. # CX1725-1, GR ACS) were used without further purification. A hydrothermal method was used to synthesize ~5 nm ceria aqueous suspension. Briefly, a 20 ml aqueous mixture of 0.01 mol cerium chloride and 0.01 M citric acid was added to 20 ml of 3 M ammonium hydroxide. After stirring for 24 h at 50 °C, the solution was transferred into a Teflon-lined stainless steel bomb and heated at 80 °C for 24 h to complete the reaction. The final dispersion of ceria ENM was infused intravenously to the rats over 1 h without any further treatment or purification.

2.2. Ceria characterization

The details of ceria ENM characterization are published in our earlier study (Hardas et al., 2010). In brief, the morphology and crystallinity of the ceria was evaluated using a 200-keV field emission analytical transmission electron microscope (JEOL JEM-2010F, Tokyo, Japan) equipped with an Oxford energy dispersive X-ray spectrometer. The particle size distributions (PSDs) were