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Zinc oxide nanoparticles induce eosinophilic airway inflammation in mice

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HIGHLIGHTS

- ZnO nanoparticles induced eosinophilic airway inflammation in a murine asthma model.
- ZnO nanoparticles without allergen application induced the expression of Th2 cytokines.
- Zinc ions did not induce eosinophilia in the BALF of mice.

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) have been widely used in industry. The metal composition of $PM_{2.5}$ might contribute to the higher prevalence of asthma. To investigate the effects of ZnO NPs on allergic airway inflammation, mice were first exposed to different concentrations of ZnO NPs (0.1 mg/kg, 0.5 mg/kg) or to a combination of ZnO NPs and chicken egg ovalbumin (OVA) by oropharyngeal aspiration on day 0 and day 7 and then were sacrificed 5 days later. The subsequent time course of airway inflammation in the mice after ZnO NPs exposure was evaluated on days 1, 7, and 14. To further determine the role of zinc ions, $ZnCl_2$ was also administered. The inflammatory cell count, cytokine levels in the bronchoalveolar lavage fluid (BALF), and lung histopathology were examined. We found significant neutrophilia after exposure to high-dose ZnO NPs on day 1 and significant eosinophilia in the BALF at 7 days. However, the expression levels of the T helper 2 (Th2) cytokines IL-4, IL-5, and IL-13 increased significantly after 24 h of exposure to only ZnO NPs and then decreased gradually. These results suggested that ZnO NPs could cause eosinophilic airway inflammation in the absence of allergens.

1. Introduction

Zinc oxide nanoparticles (ZnO NPs) are widely used in the manufacture of paints, coatings, cosmetics, sunscreens, and personal hygiene products as well as in biomedicine. The adverse health effects of zinc oxide were first described as metal fume fever in workers exposed to welding fumes or ZnO particles [1,2]. A previous study indicated that zinc exposure might be a cause of occupational asthma [3]. Adamson et al. found that among metals and metal oxide nanoparticles present in air pollution, zinc might be a crucial factor in lung inflammation [4].

Atmospheric zinc is emitted from incinerators, motor vehicles, and various industries, and it exists primarily in an oxidized form (i.e., ZnO) [5]. The size of aerosolized ZnO particles is determined by the emission source, and most particles are sufficiently small to be inhaled [6]. In an animal study, inhalation of occupationally relevant ZnO NPs caused neutrophilic inflammation in the lungs of rats [7]. Infusing ZnO NPs into mice increased the concentrations of eotaxin and interleukin-13 (IL-13) in the bronchoalveolar lavage fluid (BALF), and eosinophils were recruited to the alveolar interstitium [8].

Asthma is a chronic inflammatory disease of the airways and is the most common chronic disease of children. As shown in related

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studies, asthma is a worldwide problem. The global prevalence rates of asthma range from 1% to 18% of the population of a given country [9]. The International Study of Asthma and Allergies in Childhood showed that the prevalence of asthma symptoms varies significantly worldwide, even within genetically similar groups [10]. This finding suggests that environmental factors could be responsible for some of this variation.

There has been abundant epidemiologic evidence that air pollution can exacerbate asthma [11–14]. However, whether air pollution contributes to asthma onset remains uncertain. The Southern California Children's Health cohort study suggested that exposure to air pollution might be related to new-onset asthma [15]. A cross-sectional epidemiological study in Germany suggested that the composition of particulate matter might contribute to the higher asthma prevalence in that area [16]. Although it remains unclear which component of PM is responsible for certain biological effects, it was shown that high ambient air $PM_{2.5}$ zinc levels in combination with other components were associated with increased pediatric asthma morbidity [17].

Although many inflammatory pathways are involved in the development of asthma, eosinophils play an important role in the pathogenesis of the disease [18]. In an in vitro study, ZnO NPs had immunological effects on mouse antigen-presenting cells [19]. A previous animal study suggested that ZnO has an adjuvant effect on the Th2, but not the Th1, immune response [20].

Although epidemiological and animal studies have suggested that ZnO exposure might be related to asthma development, there have been no animal experiments to determine the causative role and pathological mechanisms of ZnO in asthma. We hypothesized that exposure to ZnO NPs would cause allergic airway inflammation in mice or would have an adjuvant effect. In the present study, we evaluated the effects of ZnO NPs on allergic airway inflammation.

2. Materials and methods

2.1. Animals

Seven-week-old female Balb/c mice (19–21 g) obtained from the National Laboratory Animal Center (Taipei, Taiwan) were maintained under a 12 h light/dark cycle at a constant temperature of $22 \pm 2\,^{\circ}C$ in $55 \pm 10\%$ relative humidity throughout the study. The animals were housed in plastic cages and had ad libitum access to LabDiet 5001 (PMI Nutrition International, Brentwood, MO, USA) food and water. The animals were acclimated for one week prior to initiation of the study. All of the animal experiments were approved by the Laboratory Animal Center of the National Taiwan University and Institutional Animal Care and Use Committee (IACUC), Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (Taiwan).

2.2. Preparation of ZnO NPs and $ZnCl_2$ solutions

To minimize the aggregation of ZnO NPs, phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) (Gibco Life Technologies, USA) by weight was used as the vehicle control (VEH) as described in previous experiments [21]. ZnO NPs (average diameter <50 nm; average surface area 10.8 m^2/g) were purchased from Sigma–Aldrich (St. Louis, MO, USA). ZnO NPs were weighed using an analytical mass balance and then suspended in VEH at a final concentration of 40 μg/mL (0.1 mg/kg; low-dose) or 200 μg/mL (0.5 mg/kg; high-dose). The suspensions were shaken and sonicated for 30 min to ensure adequate mixing and stored at 4 °C. To determine the free Zn^{2+} effect, $ZnCl_2$ was purchased from Sigma–Aldrich and was prepared in PBS to match the equivalent Zn^{2+} concentration in the 0.5 mg/kg dose of ZnO NPs. The ZnO NPs and $ZnCl_2$ solution were sonicated for 10 min and then were vortexed before oropharyngeal aspiration.

2.3. Characterization of ZnO NPs

The prepared samples were shaken and filtered through 0.1 μm filters to remove any particulates from the samples and were warmed to room temperature for at least 1 h. The hydrodynamic diameter and zeta potential of the ZnO NPs were determined using Malvern dynamic light scattering (DLS; Malvern Zetasizer Nano ZS, UK) at the Center for Nano Science and Technology, National Taiwan University. To determine the ZnO NPs morphology, the prepared solutions were carbon coated to an average thickness of 10 nm using a sputter coater (Bio-Rad, UK). The morphologies of the ZnO NPs were observed using a Nova NanoSEM 230 (FEI, Hillsboro, OR, USA) at an accelerating voltage of 5 kV with a 3.0 spot size.

2.4. Experimental design

2.4.1. Experiment A. The role of different concentrations of ZnO NPs with and without OVA on airway inflammation

The mice were divided into the following six groups: VEH; OVA; low-dose ZnO NPs (0.1 mg/kg); high-dose ZnO NPs (0.5 mg/kg); low-dose ZnO NPs + OVA; and high-dose ZnO NPs + OVA. OVA derived from chicken egg is a frequently used allergen that induces a robust allergic inflammation [22]. On days 0 and 7, the mice were exposed to 50 μL of the appropriate solution by oropharyngeal aspiration under light anesthesia (Sevoflurane; Abbott Laboratories, UK). Briefly, each mouse was held vertically, and the tongue was gently pulled out of the mouth with forceps. The solution (50 μL) was pipetted to the oropharynx when the mouse's nose was closed. By forcing the mouse to breathe through the mouth, the solution was aspirated to the lower airways [23]. For the ZnO NPs + OVA group, Balb/c mice were treated with 50 μL of ZnO NPs 1 h before they were treated with 10 μg/50 μL OVA. The animals were sacrificed 5 days after the second treatment. Tissue samples, including blood, bronchoalveolar lavage fluid (BALF) and lung, were collected (Fig 1A).

2.4.2. Experiment B. Time course of inflammatory events following ZnO NPs and zinc ion exposure

To evaluate the time course of the consequent allergic inflammation and further determine the role of zinc ions on allergic airway inflammation, we conducted a separate experiment. The mice were sensitized with VEH, OVA, low-dose ZnO NPs (0.1 mg/kg), high-dose ZnO NPs (0.5 mg/kg), or $ZnCl_2$ on days 0 and 7 and then sacrificed at 24 h, 1 week, or 2 weeks after exposure (Fig 1B).

2.5. Determination of total serum IgE

We obtained blood samples from the facial veins of the mice. Total serum IgE was measured using a Mouse IgE ELISA Kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, a predetermined concentration of capture anti-IgE Ab was coated onto ELISA plates, and the plates were incubated at 4 °C overnight. The solution was aspirated, and the plates were washed 3 times with buffer solution; subsequently, diluted serum (50×) was added, and the plates were incubated at room temperature for 2 h. The working detector solution was added, and the plates were incubated at room temperature for 1 h. Enzyme activity was evaluated using tetramethylbenzidine (TMB) and hydrogen peroxide as substrates and monitored on an ELISA reader (BioTek Instruments, Inc., VT, USA) at 450 nm with λ correction at 570 nm within 30 min of adding the stop solution. The absorbance readings were corrected to ng/mL using values obtained from standard curves.