Li et al. Particle and Fibre Toxicology (2017) 14:53
DOI 10.1186/s12989-017-0234-0

Particle and Fibre Toxicology

RESEARCH Open Access

Suppression of PTPN6 exacerbates aluminum oxide nanoparticle-induced COPD-like lesions in mice through activation of STAT pathway

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Abstract

Background: Inhaled nanoparticles can deposit in the deep lung where they interact with pulmonary cells. Despite numerous studies on pulmonary nanotoxicity, detailed molecular mechanisms of specific nanomaterial-induced lung injury have yet to be identified.

Results: Using whole-body dynamic inhalation model, we studied the interactions between aluminum oxide nanoparticles (Al_2O_3 NPs) and the pulmonary system in vivo. We found that seven-day-exposure to Al_2O_3 NPs resulted in emphysema and small airway remodeling in murine lungs, accompanied by enhanced inflammation and apoptosis. Al_2O_3 NPs exposure led to suppression of PTPN6 and phosphorylation of STAT3, culminating in increased expression of the apoptotic marker PDCD4. Rescue of PTPN6 expression or application of a STAT3 inhibitor, effectively protected murine lungs from inflammation and apoptosis, as well as, in part, from the induction of chronic obstructive pulmonary disease (COPD)-like effects.

Conclusion: In summary, our studies show that inhibition of PTPN6 plays a critical role in Al_2O_3 NPs-induced COPD-like lesions.

Keywords: Aluminum oxide nanoparticles; PTPN6, Experimental COPD, Inflammation

Background

Airborne nanoparticle-induced pulmonary toxicity has been widely reported. This toxicity is largely dependent on the chemical and physical characteristics of specific nanoparticles. Pulmonary inflammation plays a critical initial event in metal oxide nanoparticle-induced respiratory disorders [1–3].

Aluminum oxide nanoparticles (Al_2O_3 NPs) have been widely used in the chemical, industrial and medical fields [4]. Al_2O_3 NPs represent one of the most abundantly produced nanosized particles, accounting for approximately 20% of the 2005 world market of nanoparticles [5]. The limit values for inhaled aluminum (Al) compounds remain relatively high. For instance, the United States Occupational Safety and Health Administration (OSHA) has set a legal limit (PEL) of 15 mg/m^3 (total dust) and 5 mg/m^3 (respirable fraction) for alumina in dusts averaging over an 8 h work day [6]. In total alumina dusts, nano-scaled particles exhibit a slower precipitation in air than the bulk, therefore increasing exposure duration in humans [7]. It is noteworthy that asthma has been recognized as one of the most prevalent pulmonary diseases in the aluminum exposure occupational setting [4]. In addition, insoluble Al compounds appear to be biopersistent in lung tissues. As demonstrated in rat intratracheal instillation model, only ~9% of Al_2O_3 was cleared from the lungs during a 19-week period following weekly instillation for 20 weeks [8]. Considering the high discharge levels during the

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manufacturing process [7], concern over the biosafety of Al_2O_3 NPs is warranted.

In term of toxic mechanisms, exposure to Al_2O_3 NPs has been shown to cause mitochondrial dysfunction in a dose-dependent manner in human fetal lung fibroblasts (HFL1) [9] and human bronchial epithelia HBE [10]. Other studies have shown the number of macrophages in bronchoalveolar lavage fluid (BALF) of Al_2O_3 nanowhisker-exposed mice to be two-fold higher than in control mice [11]. A series of studies by Pauluhn have further suggested pulmonary inflammation induced by high dose (28 mg/m^3) Al exyhydroxides nanoparticles exposure [12, 13]. Taken together, these studies suggest a possible toxic mechanism involving oxidative stress and inflammation; however, detailed molecular mechanisms by which Al_2O_3 NPs interact with the pulmonary system have yet to be defined.

Our previous in vitro study suggested that suppression of protein tyrosine phosphatase, non-receptor type 6 (PTPN6) expression level in A549 cells plays a key role in Al_2O_3 NPs-induced cellular toxicity [14]. PTPN6 has been shown to be a critical negative regulator of intracellular signaling; it is predominantly expressed in hematopoietic cells and epithelia [15, 16]. Suppression of PTPN6 augments oxidative stress and exacerbates chronic inflammatory airway diseases [17]. Activation of the STAT3 pathway is involved in development of pulmonary inflammatory disease [18, 19], and PTPN6 has been recognized in multiple cell lines as a negative regulator of STAT3 [20–22]. In addition, up-regulation of PTPN6 has been shown to inhibit phosphor-STAT3 and mitigate activation of STAT3 in A549 cells [23]. Accordingly, we hypothesized that aberrant expression of PTPN6 might be involved in pulmonary disorders induced by Al_2O_3 NPs in vivo. To address this hypothesis, mice were exposed to Al_2O_3 NPs by dynamic inhalation to observe the alterations in lung function and pathology. We subsequently delineated the function of PTPN6 in Al_2O_3 NPs-related pulmonary disorders by integrating cellular assays with experimental mouse models. Notably, we show that rescue of PTPN6 expression levels significantly alleviates Al_2O_3 NPs-induced pulmonary inflammation as well as COPD-like lesions in murine lung tissues.

Results

Al_2O_3 NPs exposure increases levels of inflammatory mediators in mouse lung tissues

Specific airway resistance (sRAW) (an index used to identify acute bronchial response [24]) was measured in conscious mice one day before Al_2O_3 NPs-exposure and on exposure days 3 and 7. The sRAW in 2 mg/m^3 Al_2O_3 NPs-treated mice was significantly increased on day 3 and day 7 of exposure. In mice exposed to 0.4 mg/m^3 Al_2O_3 NPs, a significant increase in sRAW was noted on exposure day 7 when compared with FRA control (Fig. 1a). Based on the findings that low levels of Al_2O_3 NPs exposure caused phenotype alteration on the 7th day of exposure, all the following experiments were carried out for 7 days.

The aluminum (Al) burdens in lungs of mice were 411 ± 68, 1238 ± 110 and 2951 ± 234 ng/g for the control (filtered-room air, FRA), 0.4 and 2 mg/m^3 Al_2O_3 NPs-exposed groups, respectively. The 2 mg/m^3 Al_2O_3 NPs-exposed groups had a 3-fold greater Al burden in the lungs compared to those exposed to 0.4 mg/m^3 Al_2O_3 NPs. This may be related to greater damage to the lung in response to the higher dose, reflecting altered aggregation and deposition patterns.

Compared to FRA exposed mice, Al_2O_3 NPs-exposed mice had higher concentrations of bronchoalveolar lavage fluid (BALF) interleukin (IL)-6 (a cytokine associated with decline in lung function [25]) and BALF IL-33 (a cytokine induces chronic airway inflammation [26, 27]) (Fig. 1b). Next, we assayed the total number of cells, number of monocytic cells and neutrophils in BALF. Compared with controls, the percentages of increased levels of IL-6 were 76% and 113%; increased IL-33 levels were 65% and 72.5; increased total cell numbers were 33% and 64%; increased monocytic cells were 34% and 57%, increased numbers of neutrophils were 130% and 250% in the 0.4 and 2 mg/m^3 Al_2O_3 NPs exposed murine lungs, respectively. All of the above airway inflammatory indices were elevated in an Al_2O_3 dose-dependent manner when compared to FRA controls (Fig. 1c). Figure 1 d to g depict representative images of inflammatory infiltration in murine lung tissues, noting infiltration of inflammatory cells around small airways (Fig. 2d) (shown by arrow). Figure 1e showed the representative image of alveolar area of control murine lung. Increased infiltrating alveolar neutrophil, lymphocytes and macrophages (Fig. 1f) in response to Al_2O_3 NPs were also observed (shown by arrows).

Al_2O_3 NPs exposure induce apoptosis and experimental COPD in mice

The pathological alterations in murine lung tissues were examined by hematoxylin and eosin (H&E) staining. We noted emphysema in the distant alveolar area, characterized by enlarged airspace and increased mean chord lengths (Lm) (established indices of experimental COPD [28]) in lung tissues after Al_2O_3 NPs exposures compared to FRA control (Fig. 2a and b). The other vital characteristic of experimental COPD, small-airway remodeling, was assayed by Periodic Acid-Schiff (PAS) [27] and Masson's Trichrome staining [29]. Increased mucin glycoprotein secretion was detected by Periodic Acid-Schiff (PAS) staining, and mucus hypersecretion

Zinc oxide (ZnO) nanopowders (<100 nm particle size (manufacturer's data)) were purchased from Sigma-aldrich, USA. The average diameter of zinc oxide (ZnO) nanopowders was 86.37 nm, which were suspended in DMEM medium (with 10% FBS) at a concentration of 25 μg/mL and followed 30 min ultra-sonication (Additional file 1: Figure S4c). Aggregation of ZnO NPs within 24 h is shown in Additional file 1: Figure S4d.

Carbon black (CB) nanopowder was purchased from Plasmachem GmbH, Germany, with an average primary particle size of 13 nm (manufacturer's data). When suspending in DMEM medium (with 10% FBS) (25 μg/mL) and following 30 min ultra-sonication, the average diameter of CB NPs was 15.1 nm (Additional file 1: Figure S4e). The aggregation of CB NPs in DMEM medium is shown in Additional file 1: Figure S4f.

Cell culture and lentivirus transduction

HBE or A549 cells were seeded in 6-well plates at a density of about 1×10^6 cells per well with DMEM medium with 10% FBS.

A549 cells were treated with 0, 25 or 100 μg/ml ZnO NPs or CB NPs for 24 h, mRNA were then collected for qRT-PCR analysis.

PTPN6 overexpression lentivirus was generated by co-transfection with packaging plasmids, pSPAX2 and pMD2G. The overexpression lentivirus harbored a target gene coding sequence, which was tagged with c-Myc. A549 or HBE cells were added with lentivirus (MOI = 30) and treated with Blasticidin S for two weeks to obtain a stable transduction A549 or HBE cells. For experiments, A549 or HBE cells were thawed and allowed to grow for three passages before use. The control A549 or HBE cells and lentivirus stable transduction (LST) cells were then treated with 0 (vehicle) or 25 μg/ml Al_2O_3 NPs for 24 h, mRNA and proteins were collected for further analysis.

Animals

Male C57BL/6 mice (20–22 g) were purchased from SLRC Laboratory Animal Co., Ltd., China. Animals were treated humanely and all experimental protocols were approved by Committee on Animal Use and Care of Southeast University, China. All the methods in the present study were performed according to approved guidelines. Five mice were housed in each polycarbonate cage with ad libitum access to food and water. Light cycles were set on a 12/12 h light/dark cycle, and room temperature was set at 22.5 °C.

Animal experiments

The dynamic inhalation exposure chambers were outfitted with air quality monitoring equipment and a dust aerosol generator (Beijing HuiRongHe Technology Co., Ltd., China). The dry Al_2O_3 nanopowder was stored in sample reservoir, and the concentration of dust aerosol was adjusted by the rotation speed of the rotary brush outfitted in the dust aerosol generator (HRH-DAG768, Beijing HuiRongHe Technology, Co. Ltd., China). Exposure was carried out in stainless-steel Hinners-type whole-body inhalation chambers; the treatment groups received Al_2O_3 NPs, and the control received high efficiency particulate air (HEPA)-filtered room air (FRA) at the same flow rate as the experimental group. The mass concentrations of Al_2O_3 NPs aerosol in the whole-body chamber were measured by a real-time light scattering dust monitor (CEL-712 Microdust Pro, CASELLA CEL Inc., USA), which was placed at the same height as the top of the animal cages. The dust monitor was calibrated using a gravimetric approach by pulling Al_2O_3 NPs aerosol through a filter and weighing filter before and after, then dividing mass by the volume of air sampled. The mice were exposed to each chamber for 8 h per day (from 9 a.m. to 5 p.m.) for 7 consecutive days and sacrificed 24 h after the 7-day-treatment. Light cycles were set on at 12/12 h light/dark cycle. The temperature in the chambers was set at 22.5 °C.

The first batch of animal experiments included three groups (with 15 C57BL/6 mice in each group): control exposed to filtered room air (FRA); mice exposed to Al_2O_3 NPs with a mean concentration of 0.4 mg/m^3; or mice exposed to Al_2O_3 NPs with a mean concentration of 2 mg/m^3. The selection of exposure concentration took into account results from a previous inhalation study with Al oxyhydroxides nanoparticles [12], OSHA regulation [6], as well as our previous study [14]. Mice were exposed to Al_2O_3 NPs for 7 concecutive days and sacrified 24 h after Al_2O_3 NPs exposure. The specific airway resistance (sRAW) was measured after dynamic inhalation on exposure days 3 and 7.

The second batch of mice included two groups (with 10 C57BL/6 mice in each group): control exposed to filtered room air (FRA) and mice exposed to ZnO NPs with a mean concentration of 0.4 mg/m^3. Mice were exposed to ZnO NPs for 7 concecutive days and sacrified 24 h after ZnO NPs exposure. One piece of lung tissues were fix in 4% PFA and the other lung tissues were stored in liquid nitrogen.

The third batch of mice were divided into four groups (with 10 C57BL/6 mice in each group): control mice treated with FRA and control vector lentivirus; mice treated with FRA and PTPN6 vector lentivirus; mice treated with 0.4 mg/m^3 Al_2O_3 NPs and control vector lentivirus; and mice treated with 0.4 mg/m^3 Al_2O_3 NPs coupled with PTPN6 vector lentivirus. Mice received intranasal treatment with 1×10^8 transducing units (TU)/mouse every three days. Treatment began two days before Al_2O_3 NPs exposure. Mice were exposed to Al_2O_3