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Nanoparticles in the lungs of old mice: Pulmonary inflammation and oxidative stress without procoagulant effects

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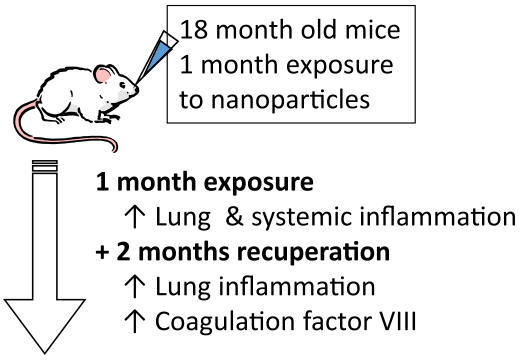
HIGHLIGHTS

- Health effects due to ultra-fine (nanoparticles) might be aggravated in elderly.
- We exposed 18-month-old C75Bl/6 mice during 5 consecutive weeks.
- The NPs caused pulmonary toxicity in these 18-month-old mice (without oxidative stress).
- Coagulation factor VIII was greatly increased up to 8 weeks after the subacute NP exposure.
- ZnO NPs induced a fibrosis-like pathology.

GRAPHICAL ABSTRACT



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ABSTRACT

Pulmonary exposure to nanoparticles (NPs) has been shown to induce pulmonary as well as cardiovascular toxicity. These effects might be enhanced in elderly subjects as a result of a compromised immunity and/or declined organ functions. To study the adverse in vivo effects of NPs in a model for the elderly, we exposed 18-month-old C75Bl/6 mice to multi-walled carbon nanotubes (MWCNTs) or ZnO NPs by intratracheal instillation once a week during 5 consecutive weeks. Pulmonary and hemostatic toxicity was determined 24 h (T1) and 8 weeks (T2) after the last administration.

Both NP types significantly increased the pulmonary macrophages at both time points. The MWCNTs and ZnO NPs also induced a pulmonary influx of neutrophils, which was even larger at T2 compared to T1. All NPs induced only a modest increase of pulmonary IL-1β, IL-6 and KC levels. Both types of NPs also increased blood neutrophils. Red blood cells were not significantly affected. Both NPs significantly increased coagulation factor VIII levels at both time points. Histological analysis revealed the presence of MWCNTs in the alveolar macrophages up to 8 weeks after the last administration and the ZnO NPs induced a pronounced alveolar inflammation.

Abbreviations: aPTT, activated partial thromboplastin time; BAL, broncho-alveolar lavage; BSA, bovine serum albumin; CRP, C-reactive protein; DAB, 3,3′-diaminobenzidine; ENPRA, engineered nanoparticle risk assessment; GSH, reduced glutathione; GSSG, oxidized glutathione; HBSS, Hank's balanced salt solution; IFN-γ, interferon gamma; IL, interleukin; KC, cytokine-induced neutrophil chemo-attractant; LAL, limulus amoebocyte lysate; LPS, lipopolysaccharide; MCV, mean corpuscular volume; MPV, mean platelet volume; MWCNT, multi-walled carbon nanotube; NP, nanoparticle; PT, prothrombin time; RBC, red blood cell; T1, time point 1 = 24 h after the last instillation; T2, time point 2 = 8 weeks after the last instillation; TNF-α, tumor necrosis factor alpha; WBC, white blood cell; ZnO, zinc oxide.

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In these 18-month-old mice, NPs caused pulmonary inflammation (without evidence of oxidative stress) accompanied by large increases in coagulation factor VIII up to 8 weeks after the last NP exposure. The persistence of the MWCNTs in the lungs resulted in translocation from the lungs to the left heart and the ZnO NPs induced a fibrosis-like pathology.

1. Introduction

Nanotechnology is currently applied in various industries for manufacturing novel consumer products, such as cosmetics, clothing or sporting goods, … Nanomaterials are also under study for use in medicine (Qian et al., 2017; Saptarshi et al., 2015). Almost half of the nanoparticle (NP)-containing products originate from the USA (Nanotechnology, n.d.). The use of NP-containing products may lead consumers to be exposed to NPs released from the product matrix due to the natural wear and tear. Inhalation is the most likely route of exposure, although oral uptake is also possible (Upadhyayula et al., 2014).

Despite the numerous benefits of these novel applications, a lot of research suggests toxicity after NP exposure. Several animal studies have shown pulmonary toxicity after the inhalation, instillation or aspiration of different types of NPs such as acute lung injury, increased risks of pulmonary fibrosis and cancer (Card et al., 2008; Oberdorster et al., 2005). It is generally believed that the mechanisms underlying this toxicity are inflammation and oxidative stress (Marano et al., 2010). However, the targets of toxicity induced by NP inhalation are not limited to the lungs, systemic effects and cardiovascular toxicity should also be considered. In animal models, the pulmonary administration of engineered NPs has been shown to induce endothelial dysfunction, promote atherosclerosis and to have pro-coagulant effects (Geys et al., 2008; LeBlanc et al., 2010; Nemmar et al., 2005; Nurkiewicz et al., 2008). Possible pathways linking the pulmonary and cardiovascular compartments consist of 1/ translocation of particles from the lungs to the systemic circulation thus exerting direct effects on the heart, blood and vasculature; 2/ the release of mediators (pro-inflammatory cytokines, oxidatively-modified macromolecules, vasoactive proteins, and prothrombotic factors) from the lung to the cardiovascular compartment and 3/ the stimulation of sensory nerves in the lung thus altering cardiac rhythm and function (Shannahan et al., 2012; Brook et al., 2010).

So far, most studies have used young healthy animals to assess the NP effects. However, some groups within the general population may be more susceptible to the deleterious effects of NPs and suffer greater damage from the same exposure than others. This includes children, people with cardiovascular disease (congestive heart failure, chronic coronary artery disease), chronic respiratory diseases, diabetics and the elderly (>65 years) (Goldberg et al., 2000; Gouveia and Fletcher, 2000; Jacobs et al., 2010; Jacobs et al., 2012). Increased sensitivity in older age can be explained by alterations in the neuroendocrine network, compromised immunity and a general decline in cellular and organ functions. Therefore, NPs may exhibit unusual absorption, distribution, metabolism and excretion profiles; and impaired protective/repair functions in elderly subjects may lead to worse toxic consequences than for healthy populations. Also, weaker anti-oxidative and immune functions may magnify toxicity (Li et al., 2014).

In this study, we focus on the elderly because the incidence of thrombotic cardiovascular disease increases with age (Tracy and Bovill, 1992). Epidemiological research has consistently demonstrated associations between episodes of increased air pollution and daily morbidity and mortality. Short-term, as well as long-term exposures to increased levels or air pollutants have been associated with cardiac arrhythmias, increases in blood pressure, subclinical atherosclerosis, deep vein thrombosis (Pope III and Dockery, 2006). Although limited epidemiological evidence data is available for ultrafine particles (<0.1 μm), experimental evidence indicates that this size fraction poses a high risk for the cardiovascular system (Brook et al., 2010). Age-related changes occur in the hemostatic system which involves platelets, coagulation and fibrinolytic factors, and in the vasculature where sclerotic changes contribute to an increased incidence of thrombosis in the elderly (Kiechl and Willeit, 1999). In other words, the blood of the elderly is in a state of hypercoagulability due to an increased platelet activity and increased plasma levels of several coagulation factors without a proportional increase in anticoagulant factors (Balleisen et al., 1985; Sagripanti and Carpi, 1998). Increased levels of coagulation factor (F) VII, FVIII and fibrinogen have been shown to be associated with an increased risk for thrombotic disease (Koster et al., 1994).

To evaluate the pulmonary and hemostatic toxicity of NPs in a model with relevance to the elderly population, we repeatedly exposed 18-month-old mice to multi-walled carbon nanotubes (MWCNTs) or zinc oxide (ZnO) NPs. Either 24 h or 8 weeks after the last administration, pulmonary inflammation and oxidative stress were determined together with blood cell counts and several hemostasis parameters.

2. Materials and methods

2.1. Animal model

Eighteen-month-old male C57Bl/6 mice weighing, on average, 32.1 g at the start of the experiment were purchased from Janvier (Le Genest-Saint-Isle, France).

All animals were kept in micro-isolation cages in a temperature- and light-controlled (12-hour night/day cycle) environment and had free access to drinking water and standard chow (KM-04-k12, Muracon, Carfil, Oud-Turnhout, Belgium; 13% kcal as fat, caloric value 10.9 kJ/g) ad libitum. All animal procedures were approved by the Ethical Committee of the Katholieke Universiteit Leuven and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996).

2.2. Nanomaterials and preparation of exposure suspensions

The MWCNTs (Table 1) were purchased from Arkema Graphistrength C100 (NM402; diameter 30 nm, length 20 μm), ZnO NPs manufactured by BASF Z-Cote (NM 110; zinkite, uncoated, 100 nm) were received from the European Joint Commission Joint Research Centre (Ispra, Italy). A detailed characterization has been published earlier (Kermanizadeh et al., 2013; Luyts et al., 2014).

All NPs were weighed and dispersed in sterile distilled water containing 2 vol% mouse serum (dispersion medium) to create a stock concentration of 2.56 mg/ml. The mouse serum was obtained from healthy mice via puncture of the inferior vena cava. The blood was collected in Minicollect serum tubes, left at room temperature for 30 min and centrifuged at 2000g during 10 min. The supernatant (serum) was collected and tested to be free of LPS (LAL assay). In sterile conditions, the mouse serum was diluted to 2 vol% in Baxter water. The NP stock suspensions were sonicated using a Microson™ ultrasonic cell disruptor (Misonix, Newtown, USA), equipped with a 1/8″ disruptor horn for 16 min on ice/water. Thereafter, the stock concentrations were diluted to obtain the final concentrations for exposure: 512 μg/ml MWCNT (corresponds to 25.6 μg/instillation) and 256 μg/ml ZnO (12.8 μg/instillation). All NP suspensions were used within 1 h after sonication.