Original Article

Pulmonary Toxicity in Rats Caused by Exposure to Intratracheal Instillation of SiO_2 Nanoparticles*

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Abstract

Objective The effect of the silica nanoparticles (SNs) on lungs injury in rats was investigated to evaluate the toxicity and possible mechanisms for SNs.

Methods Male Wistar rats were instilled intratracheally with 1 mL of saline containing 6.25, 12.5, and 25.0 mg of SNs or 25.0 mg of microscale SiO_2 particles suspensions for 30 d, were then sacrificed. Histopathological and ultrastructural change in lungs, and chemical components in the urine excretions were investigated by light microscope, TEM and EDS. MDA, NO and hydroxyproline (Hyp) in lung homogenates were quantified by spectrophotometry. Contents of TNF-α, TGF-β1, IL-1β, and MMP-2 in lung tissue were determined by immunohistochemistry staining.

Results There is massive excretion of Si substance in urine. The SNs lead pulmonary lesions of rise in lung/body coefficients, lung inflammation, damaged alveoli, granuloma nodules formation, and collagen metabolized perturbation, and lung tissue damage is milder than those of microscale SiO_2 particles. The SNs also cause increase lipid peroxidation and high expression of cytokines.

Conclusion The SNs result into pulmonary fibrosis by means of increase lipid peroxidation and high expression of cytokines. Milder effect of the SNs on pulmonary fibrosis comparing to microscale SiO_2 particles is contributed to its elimination from urine due to their ultrafine particle size.

Key words: Silica (SiO_2) nanoparticles; Collagen synthesis; Lipid peroxidation; Cytokines; Pulmonary toxicity

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INTRODUCTION

Application of synthetic silica nanoparticles (SNs) has received wide research attention in a variety of industries. SNs are produced on an industrial scale as additives for cosmetics, drugs, printer toners, varnishes, and food. In addition, the SNs are being developed for different biomedical and biotechnological applications, such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization[1-3]. With growing commercialization of nanotechnology products, the chance for human exposure to SNs is increasing and many aspects

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related to these nanomaterials have raised concerns about their safety[4-13]. Contrary to the well-studied crystalline micron-sized silica, relatively little information exists on their toxicity. Because nanoparticles possess novel properties, like kinetics and unusual bioactivity, their potential biological effects may differ greatly from those of micron-sized bulk materials. Most of the *in vitro* studies on the SNs have reported results for their cellular uptake, size- and dose-dependent cytotoxicity, increased reactive oxygen species levels and pro-inflammatory stimulation[5-11,14-16]. Evidence from a limited number of *in vivo* studies on the SNs demonstrates largely reversible lung inflammation, granuloma formation and focal emphysema after their exposure, with no progressive lung fibrosis[12-13,17-19]. Reliable *in vitro* assays are currently not available for predicting the full effects of the nanoparticles on the lung tissue[20-22]. More research with standardized materials is therefore needed to enable comparison of experimental data for different forms of SNs, to establish which physicochemical properties are responsible for their observed toxicity. *In vivo* models are thus necessary to study the effect of the SNs, because high permeability through the air-blood barrier allows their fast uptake.

The aim of this present study is therefore to study and clarify biological and pathological events on intratracheally instilled SNs in the rats' lungs during sub-acute stage, using histopathological and ultrastructural evaluations. In addition, factors, such as oxidative stress and pro-inflammatory that could be important in the induction of pulmonary toxicity by the SNs are comparatively investigated using colorimetry and immunohistochemistry.

MATERIALS AND METHODS

Materials

Two sized SiO_2 powders were used in the experiments. SNs were provided by Zhejiang Hongshen Material Technology Limited Company (Zhejiang, China). Silica content in the SiO_2 nanoparticles is more than 99.5% and the hydroxyl group content on the surface of the SiO_2 nanoparticles is more than 45%. The surface area for the SNs is 640 ± 30 m^2/g (as provided by the production company). Microscale SiO_2 powder is obtained from Sigma-Aldrich (Cat. no. 5631, USA), approximately 80% of the microscale SiO_2 particles has diameter between 1-5 μm, and quartz purity was 99%, according to the data sheet of the company.

Suspensions of sterilized SNs and microscale SiO_2 particles were diluted to different saline concentrations and then mixed with 20,000 U penicillium. The suspensions were then dispersed by ultrasonic vibration for 15 min and shaken vigorously by a vortex shaker prior to administration.

Animal Treatment

All animal experiments were performed in compliance with local ethics committee. Specific pathogen-free male Wistar rats (180-220 g weight) were purchased from Zhejiang Research Center for Experimental Animals (Zhejiang, China) at 7th week of age. They were then acclimated for 3 d during which each animal was examined to confirm suitability for study. The rats were kept in individual cages and had free access to food and water. The animals were maintained in controlled environmental temperature (25 ± 1 °C), relative humidity (45% ± 5%) and 12 h light/dark circle. 10 rats in the trial experiments were divided randomly into two groups with five rats in each group. The rats were anesthetized lightly with ether which was instilled intratracheally with SNs and microscale SiO_2 powder dissolved in saline at 50.0 mg/mL concentrations. The SNs and microscale SiO_2 powder we observed *via* trachea under direct observation using a laryngoscope for 24 h. 50 rats were divided randomly into five groups with 10 rats in each group in subsequent experiments. The rats were administered, as trial experiment, with 1 mL saline containing 6.25, 12.5, and 25.0 mg of SNs suspensions or 25.0 mg of microscale SiO_2 powder suspensions.

Lung Collection and Histopathological Examination

30 d after instillation of the test material into the rats, body weights were determined in the 30 d groups. Each rat was injected intraperitoneally with a lethal dose (0.1 mL) of pentobarbital sodium solution. The lungs were then removed immediately from the thorax and weighted (lung wet weight). The lung/body coefficient was then calculated using the following equation: lung / body coefficient (%) = lung wet weight (g) / body weight (g) × 100%. A small piece of lung was fixed by 10% formalin for at least 7 d before further processing. The formalin-fixed mouse lungs were embedded in paraffin from which thin coronal sections were mounted on glass microscope slides using standard histopathological techniques. Sections were stained with hematoxylin-eosin and examined by light