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Ultrastructural changes in the air–blood barrier in mice after intratracheal instillations of Asian sand dust and gold nanoparticles

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ABSTRACT

The purpose of this study was to investigate a possible translocation pathway of intratracheally instilled gold nanoparticles after the induction of acute pulmonary injury by Asian sand dust. ICR mice were intratracheally instilled with 800 μg Asian sand particles (CJ-2 particles) 24 h before instillation of 50-nm gold nanoparticles. Lungs from mice treated with Asian sand particles and gold nanoparticles showed an acute focal inflammation with an increased expression of proinflammatory cytokines (IL-6 and TNF-α) and oxidative stress markers (Cu/Zn SOD and iNOS) in alveolar macrophages, type I alveolar epithelial cells, and endothelial cells at the alveolar walls. Electron microscopy revealed a destruction of the alveolar walls with an increased number of endocytic vesicles in the cytoplasm of both type I epithelial cells and endothelial cells; gold nanoparticles were demonstrated in these endocytic vesicles. These findings suggest that translocation of the exposed nanoparticles may be enhanced in the lung tissues with acute inflammatory changes.

1. Introduction

Asian sand dust originates from the Gobi desert and Loess plateau of China and Mongolia (Kim et al., 2010; Park et al., 2010). During the winter and spring seasons, dust storms spread and transport dust particles to far-reaching areas such as China, Mongolia (Park et al., 2010), South Korea (Chung and Yoon, 1996; Kim et al., 2010), Taiwan (Chen et al., 2004; Chang et al., 2010), Japan (Mori et al., 2003; Maki et al., 2010), the United States, and Canada (Husar et al., 2001; Tratt et al., 2001; Zdanowicz et al., 2006). Epidemiologic studies have shown that Asian dust affects human health in several regions. A concomitant increase in respiratory and cardiovascular problems has been reported in Taiwan (Yang et al., 2005), China (Meng and Lu, 2007), and Korea (Hong et al., 2010). In experimental animal models, exposure to Asian sand particles induces an acute neutrophilic inflammation in bronchi and alveoli (Ichinose et al., 2005; Naota et al., 2010) and exacerbates allergic alveolitis with goblet cell proliferation in the airways (Hiyoshi et al., 2005). Intratracheal instillation of Asian sand particles also induces inflammatory cell proliferation and increases the level of bronchoalveolar lavage fluid (BALF) chemokines and inflammatory cytokines (Ichinose et al., 2005; Naota et al., 2010).

In a normal environment, ambient air is a mixture of various types of materials (Inoue et al., 2007; Laks et al., 2008). On a daily basis, people are exposed to many kinds of substances, either on purpose or accidentally. Nanotechnology is developing rapidly and is involved in a wide range of applications. Therefore, the exposure to nanoscale materials and their potential toxicity are of great concern. Gold nanoparticles are well-known nanomaterials with multipurpose usages (Balasubramanian et al., 2010; De Jong et al., 2008; Sadauskas et al., 2009a). A number of studies have demonstrated that the translocation of gold nanoparticles into the systemic circulation is extremely low after intratracheal instillation and inhalation in physiological conditions (Lipka et al., 2010; Sadauskas et al., 2009a; Semmler-Behnke et al., 2008; Takenaka et al., 2006; Yu et al., 2007).

Seasonal or daily exposure to endemic or epidemic pollutants such as Asian sand dust results in acute pulmonary injury (Ichinose

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et al., 2005; Naota et al., 2010), and it may enhance the translocation of nanoparticles into the systemic circulation across the damaged air–blood barrier, thereby exacerbating systemic toxicity. Several reports have described the effects and the translocation process of gold nanoparticles after intratracheal instillation in healthy mice (Furuyama et al., 2009; Sadauskas et al., 2009a). There are, however, no reports of the effects of exposure to gold nanoparticles in an injured pulmonary condition. The purpose of this study was to investigate the possible translocation pathway of 50-nm gold nanoparticles in the pulmonary pathological condition induced by Asian sand dust in mice.

2. Materials and methods

2.1. Animals

Fifty-five male ICR mice (7 weeks old, 33–36 g) were obtained from CLEA Japan Inc. (Tokyo, Japan). Commercial diet CE-2 (CLEA Japan Inc.) and tap water were given *ad libitum* throughout the experiment. Mice were housed at approximately 25 °C, in 55–70% relative humidity, and in a 12-h light/dark cycle. All animal experiments were conducted according to the Tottori University guidelines for animal welfare (http://www.tottori-u.ac.jp/kouhou/kisokusyuu/reiki_honbun/u0950581001.html).

2.2. Particle preparation

2.2.1. Preparation and morphology of Asian sand particles

Simulated Asian mineral dust (CJ-2 particles) collected from Tengger Desert, China, was purchased from General Sciences Cooperation (Tokyo, Japan). The primary mineral component was composed of 28.0% Si, 5.9% Al, 5.3% Ca, and 3.0% Fe (according to the manufacturer's data sheet). A morphological examination of the CJ-2 particles was performed with a scanning electron microscope (SEM Model X-650, Hitachi, Tokyo, Japan). The particles were pleomorphic and had rough surfaces (Fig. 1A).

2.2.2. Preparation and morphology of gold nanoparticles

A 0.01% 50-nm colloidal gold solution (mean diameter, 49.3 nm; BB International, Cardiff, UK) was concentrated into a 0.1% solution by centrifugation. After centrifugation, the sediment pellets were suspended in normal saline solution. A morphological examination of the colloidal gold suspension under transmission electron microscopy (TEM-100CX, Japan Electron Optical Laboratory, Tokyo, Japan) showed electron-dense, spherical, uniform, and individual or slightly agglomerated particles (Fig. 1B).

2.3. Experimental protocol

ICR mice were divided into 2 treatment groups and 1 control group. All mice were anesthetized by intraperitoneal administration of sodium pentobarbital (5 mg/100 g body weight) before particle treatment. All mice were intratracheally instilled with 0.05 ml of solution, followed by 0.15 ml of air, with a small cannula. The suspensions were agitated immediately before instillation.

Mice were first intratracheally instilled with 800 μg CJ-2 particles (CJ-2 particles and gold nanoparticles treated group, $n = 20$) or normal saline (gold nanoparticles treated group, $n = 20$). After 24 h, mice were instilled a second time with a 0.1% 50-nm colloidal gold solution. The control mice ($n = 15$) were instilled with 0.05 ml of saline solution on both occasions.

2.4. Lungs and other organ samples

Eight mice from each of the treated groups and 6 mice from the control group were sacrificed by exsanguination under deep anesthesia induced by an intraperitoneal injection of sodium pentobarbital. At 5 min after the second instillation, the lungs were collected for histopathology, immunohistochemistry, autometallography (AMG), and transmission electron microscopy. Four mice from the treated groups and 3 mice from the control group were used for BALF collection.

At 1 h after the second instillation, mice from the treated groups ($n = 8$ per group) and the control group ($n = 6$) were sacrificed, and various organs (lungs, liver, kidneys, spleen, heart, and brain) were collected for histopathologic, autometallographic examinations, and inductively coupled plasma-mass spectroscopy (ICP-MS).

2.5. Histopathology

Lungs were fixed by infusion and immersion in 10% neutral-buffered formalin. Other tissues (liver, kidney, spleen, heart, and brain) were fixed by immersion in 10% neutral-buffered formalin. Formalin-fixed tissues were processed using routine pathological methods and embedded in paraffin blocks. Tissue sections (3-μm-thick) were cut for hematoxylin and eosin staining, immunohistochemistry, and AMG. A lung score was determined by scoring the degree of bronchiolar and alveolar inflammation (Table 1, modified from Naota et al., 2010). The histopathological examination was performed by 2 pathologists in blind manner.

2.6. Immunohistochemistry

Paraffin-embedded sections of the lungs of mice from the treated groups ($n = 8$) and the control group ($n = 6$) were used

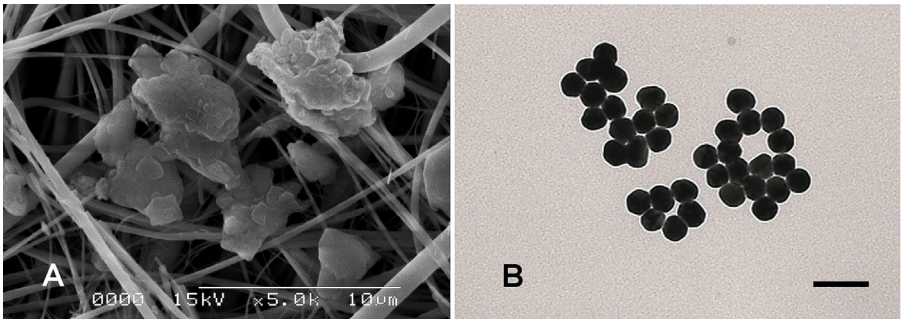

Fig. 1. Morphological findings of particles. (A) CJ-2 particles show pleomorphism with rough surfaces under scanning electron microscopy. Bar = 10 μm. (B) Transmission electron micrograph of 50-nm gold nanoparticles shows electron-dense, spherical, and uniform appearance. Bar = 100 nm.

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