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Ultrastructural changes of the air–blood barrier in mice after intratracheal instillation of lipopolysaccharide and ultrafine carbon black particles

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Abstract

Epidemiological studies have indicated associations between exposure to increased concentrations of ambient ultrafine particles and adverse health effects especially in susceptible individuals. To ellucidate the mechanisms underlying the findings from epidemiological studies, mice pretreated with lipopolysaccharide (LPS) (acute lung injury model) were intratracheally instilled with ultrafine carbon black particles (UFCB), and the air–blood barrier was observed to examine the translocation pathway of UFCB from the lung into the systemic circulation. In addition, lung toxicity induced by the intratracheal instillation of LPS and UFCB was studied with the use of electron microscope. LPS treatment induced acute inflammatory changes with increased number of activated macrophages and neutrophils in the degenerated alveolar walls. UFCB were demonstrated on or in the denuded basement membrane in the air–blood barrier; these findings were associated with edematous changes and fragmentation of the cytoplasms of alveolar epithelial cell type 1, and the damages of alveolar epithelial cell type 1 were frequently observed in the close vicinity of the clumps of UFCB. These findings suggest that translocation of the exposed ultrafine particles may be enhanced in the lung tissues with acute inflammatory changes.

Keywords: Air–blood barrier; Electron microscopy; Intratracheal instillation; Lipopolysaccharide; Lung toxicity; Ultrafine carbon black particles

Introduction

Epidemiological studies have indicated associations between exposure to increased concentrations of ambient ultrafine particles (UFPs) and adverse health effects especially in susceptible individuals (Dockery et al., 1993; Ibald-Mulli et al., 2002; Kreyling et al., 2006; Peters et al., 2006; Peters and Pope, 2002; Pope, 2004; Schulz et al., 2005; Wichmann and Peters, 2000; Wichmann et al., 2000). Indeed, peaks of ambient particulate air pollution are associated with an increase in pulmonary and cardiovascular morbidity and mortality (Nemmar et al., 2002a). However, the mechanisms remain unclear, and several mechanisms have been hypothesized.

In the real world, ambient air contains endotoxin; therefore, we simultaneously inhale endotoxin and suspended particulate matter (SPM) or inhale SPM in the lung damaged by endotoxin. In other words, we are

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involuntary primed by endotoxin. In addition, for the extrapolation to the human situation, it would be of interest to know whether an early existing and earlier-induced inflammation can be exacerbated by exposure to UFPs (Inoue et al., 2006). Previous study suggested that UFPs enhance lung inflammation related to bacterial endotoxin, lipopolysaccharide (LPS) (Inoue et al., 2006). LPS treated mice have been used as the pathological condition model (air way inflammation model) (Brigham and Meyrick, 1986); LPS activates alveolar macrophages and induces neutrophil infiltration resulting in damage of the lung tissue including the air–blood barrier (Blackwell et al., 1999). There appears to be no report of the electron microscopic study of the lung damage induced by LPS. In addition, it is suggested that UFPs are translocated from the lung into the blood circulation through the air–blood barrier, but there are few reports on the electron microscopic study of the translocation of the instilled UFPs at the air–blood barrier (Geiser et al., 2005; Shimada et al., 2006).

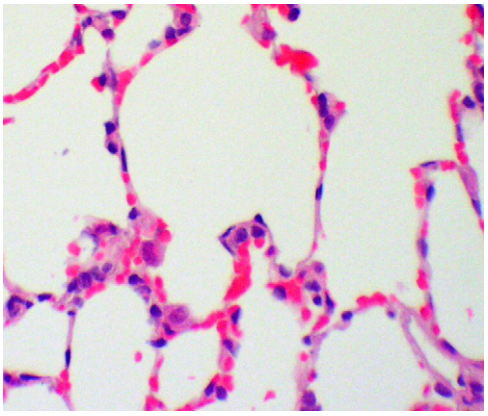

Fig. 1. Thin air–blood barrier with occasional macrophages and neutrophils were observed in the lungs from control animals. HE stain. × 400.

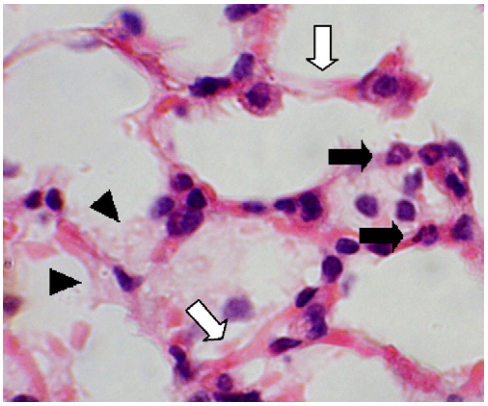

Fig. 2. Mild infiltration of neutrophils (arrows), abruption of alveolar epithelial cells type 1 (arrow heads), and thickening of alveolar wall (white arrows) were observed in the lungs from LPS treated animals. HE stain. × 400.

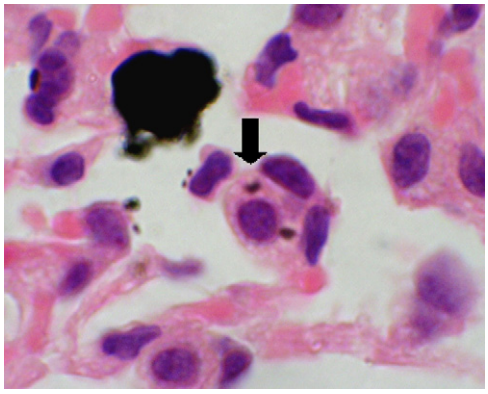

Fig. 3. UFCB (arrow) in the cytoplasm of activated macrophages were observed in the lungs from UFCB treated animals. HE stain. × 800.

The purpose of this study was to demonstrate ultrastructural changes of the air–blood barrier in mice after intratracheal instillation of LPS and ultrafine carbon black particles (UFCB).

Materials and methods

Experimental animals

Ten-week-old female ICR mice weighing 27–34 g were obtained from CLEA JAPAN Inc. (Tokyo, Japan). Animals were kept at around 25 °C and pelleted food and water were available *ad libitum* throughout the experiment. All animal experiments were performed according to the National Institute for Environmental Studies guidelines for animal welfare.

Particle and LPS suspension

UFCBs (Printex 90, 14 nm diameter) (Degussa, Frankfurt, Germany) were obtained. Particle–PBS suspensions were prepared at the concentration of 12.5 mg/ml and sterilized by autoclave at 120 °C.

LPS (*Escherichia coli* O111:B4, Chemicon International, Temecula, CA, USA)–PBS suspensions were prepared at the concentration of 150 endotoxin unit (EU)/ml.

Intratracheal instillation

The preparations were always vortexed immediately prior to the intratracheal instillation. All mice were anesthetized by an intraperitoneal injection of xylazine (Celactal; Bayer, Leverkussen, Germany), 3 mg/kg body weight and ketamine hydrochloride (Ketalal; Sankyo Pharmaceutical, Tokyo, Japan), 75 mg/kg body weights, or Nembutal 5 mg/100 g body weight.