Asian Dust Particles Induce TGF-β₁ via Reactive Oxygen Species in Bronchial Epithelial Cells

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Background: Asian dust storms can be transported across eastern Asia. In vitro, Asian dust particle-induced inflammation and enhancement of the allergic reaction have been observed. However, the fibrotic effects of Asian dust particles are not clear. Production of transforming growth factor β₁ (TGF-β₁) and fibronectin were investigated in the bronchial epithelial cells after exposure to Asian dust particulate matter (AD-PM10).

Methods: During Asian dust storm periods, air samples were collected. The bronchial epithelial cells were exposed to AD-PM10 with and without the antioxidant, N-acetyl-L-cysteine (NAC). Then TGF-β₁ and fibronectin were detected by Western blotting. The reactive oxygen species (ROS) was detected by the measurement of dichlorodihydrofluorescin (DCF), using a FACScan, and visualized by a confocal microscopy.

Results: The expression of TGF-β₁, fibronectin and ROS was high after being exposed to AD-PM10, compared to the control. NAC attenuated both TGF-β₁ and fibronectin expression in the AD-PM10-exposed bronchial epithelial cells.

Conclusion: AD-PM10 may have fibrotic potential in the bronchial epithelial cells and the possible mechanism is AD-PM10-induced intracellular ROS.

Key Words: Air Pollutants; Reactive Oxygen Species; Transforming Growth Factor β

Introduction

Fibrosis is an important process in the development of several pulmonary diseases. Airway remodeling in asthma and chronic obstructive pulmonary disease (COPD) is the result of a fibrotic reaction surrounding the airways of the lung, such as basement membrane thickening and peribronchiolar fibrosis. The etiologic factors of COPD are known to include not only cigarette smoke, but also pollutants. Furthermore, idiopathic pulmonary fibrosis (IPF) is a fatal lung disease with irreversible fibrosis. Although the diagnosis of IPF requires exclusion of other known causes of interstitial lung diseases, such as drug environmental exposure, medication or systemic disease, there are several pieces of evidence that environmental agents may have an etiologic role in pulmonary fibrosis. Inhaled environmental agents, such as smoke, dust, and fumes, have been proposed to be involved in the development of pulmonary fibrosis. Some epidemiologic studies showed that metal exposure and wood dust exposure are increased in patients with IPF. Other studies have shown that exposure to metal dust, such as cobalt, aluminium, zinc, cadmium, and mercury are associated with the development of pulmonary fibrosis.

Air pollutants have been recognized as a major problem for human health. Many epidemiologic studies have...
correlated episodes of elevated PM10 levels with increased mortality and morbidity. Air pollution is associated with a variety of cellular toxicities, including inflammation, DNA damage, and fibrosis. Exposure to air pollution particulates has been associated with airway fibrosis other than fibrotic reactions of the lung parenchyma. Asian dust is the long-range transport of atmospheric pollutants during dust events in eastern Asia, which originate in the Chinese and Mongolian deserts during spring season. Recently, there have been possible adverse effects of these dust events, as the dust storms pass through industrialized areas, such as northeastern China, which increases the probability that Asian dust contains combustion-source particles. Asian dust storm events are associated with an increase in daily mortality in Seoul, Korea, and Taipei, Taiwan. The dust also causes aggravation of respiratory symptoms of patients with airway diseases, neutrophilic airway inflammation in mice, and allergic reactions. There are several evidences that Asian sand particles could enhance inflammation by respiratory pathogens. However, there are no investigations regarding the fibrotic effect of the Asian sand particles. The present study was designed to investigate whether Asian dust particles induce production of transforming growth factor-β (TGF-β) and fibronectin through oxidative pathways in bronchial epithelial cells.

Materials and Methods

1. Asian dust particles (AD-PM10) sampling

Air samples were collected over the course of 2 years between 2004 and 2005 in Incheon City of South Korea. Asian dust particles (AD-PM10) were sampled using a high volume air sampler (HV500F; Sibata, Tokyo, Japan) with airflow at 500 L/min for at least 5 hours. For sample collection, glass microfiber filters (Millipore, Bedford, MA, USA) with a pore size of 0.25 μm were used. Filters were stored at 4°C until use. Particles were suspended in phosphate buffered saline (PBS) and sonicated the particles for 3 minutes at maximal watt, after which the materials were sieved through filters, 10 μm in size (Mitex membrane filters; Millipore). All AD-PM10 suspensions were sterilized for removal of microorganisms. Endotoxin was measured by a Limulus amebocyte lysate assay kit (BioWhittaker, Walkersville, MD, USA), according to the manufacturer’s specifications. Endotoxin levels in AD-PM10 samples analyzed within 24 hours of collection gave results similar to those obtained in stored samples and after sterilization.

2. Chemical composition of AD-PM10

Chemical composition of AD-PM10 was analyzed at the Korea Institute of Ceramic Engineering and Technology. AD-PM10 contained 48% SiO₂, 12% Al₂O₃, 5% Fe₂O₃, and 1% TiO₂ (Figure 1). There are several evidences that Asian sand particles could enhance inflammation by respiratory pathogens. However, there are no investigations regarding the fibrotic effect of the Asian sand particles. The present study was designed to investigate whether Asian dust particles induce production of transforming growth factor-β (TGF-β) and fibronectin through oxidative pathways in bronchial epithelial cells.

3. Exposure of cells to AD-PM10

Normal bronchial epithelial cells (WI-26VA4 cell line; KCLB, Seoul, Korea) were cultured in Dulbecco’s modified Eagle medium (DMEM; Gibco BRL, Gaithersburg, MD, USA) containing 10% fetal bovine serum (FBS). Cells were incubated in 5% CO₂ at 37°C. Cells were cultured in 6-well plates and allowed to grow to confluence. Near-confluent cells were incubated with DMEM containing 0.5% FBS for 24 hours. After this time period, cells were treated with prepared AD-PM10. Cells were incubated with each concentration (10, 50, 100,