ABSTRACT

Pulmonary Toxicity of Suspended Particulate matter in Mice

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Exposure to air pollutants such as suspended particulate matter (SPM) has been associated with an increase in mortality and hospital admissions due to respiratory and cardiovascular disease. With the advance of technology and environmental changes, concentration of nanoparticles and Asian sand dust (ASD) particles in ambient air has increased. Therefore, adverse health effects caused by these kinds of SPM received greater attention. In this study, lungs of mice treated with intratraheal instillation of SPM were used to elucidate the toxicity of SPM including nanoparticles and ASD.

Nanoparticles have been suggested to pose a great risk to human health due to their ability to penetrate from the lung alveoli into the blood circulation. In the first and second chapters of the thesis, the translocation mechanism of nanoparticles at the air-blood barrier (ABB) was investigated by using C60 fullerene nanoparticles and gold colloid nanoparticles. In the first chapter, histopathological and electron microscopic results showed that instilled C60 fullerene particles in alveoli, alveolar lumen and capillary lumen without any signs of injury immediately after instillation. Instilled C60 fullerene particles were observed throughout the structure of the ABB and in caveolae-like vesicles in alveolar epithelial cells (AEC). These findings suggest that C60 fullerene particles may pass the ABB by both diffusion and caveolae mediated pinocytosis resulting in immediate translocation into the systemic circulation. In the second chapter, association of endocytosis in the translocation of nanoparticles at the ABB was studied. Fifteen minutes after instillation, gold nanoparticles were observed in the cytoplasm of macrophages, on the surface of AEC and in alveoli in light microscopy. Electron microscopy demonstrated particles in vesicles of macrophages, on the surface of AEC and in the caveolae-like vesicles in AEC type 1. Immunohistochemisrty demonstrated positive immunolabeling for caveolin-1 in the ABB of

the intact lungs and lungs treated with gold particles. Double immunofluorescence and immunoelectron microscopy revealed the presence of caveolin-1 in AEC in the intact lungs of mice. These results suggest that instilled nanoparticles could be internalized into the alveolar epithelium at the ABB by caveolae-mediated endocytosis, which is regarded as physiological function of AEC.

ASD events associated with an increase in pulmonary morbidity and mortality have increased rapidly in the east Asian region since 2000. In the third and fourth chapters of the thesis, acute and chronic pathological changes caused by ASD in lung were examined. In the third chapter, acute pathological changes and its pathogenesis caused by ASD were described. Instillation of high dose of ASD particles caused acute inflammatory changes characterized by infiltration of macrophages and neutrophils around the accumulation of particles in the bronchioles and the alveoli of the lung tissues at 24 hours after instillation. Degenerated alveolar walls and bronchial epithelial cells, as well as a weakened positive immunolabeling for laminin, were observed to be associated with particle attachment. Positive immunolabelings for cytokines and oxidative stress markers were observed mainly in the inflammatory cells in the lesions. These results suggest that Asian sand dust particles caused damage to the lung tissue through a direct physical effect. In addition, secondary released cytokines and oxidative stress generated in the lesion may be involved in the development of the acute lung toxicity. In the forth chapter, chronic pathological changes caused by high dose of ASD and its pathogenesis were described. Histopathological examination revealed that ASD induced acute inflammation at 24 hours. The acute inflammation was transient and subsided at 1 week and 1 month after instillation. At 2 and 3 months after instillation, focal infiltration of lymphocytes with accumulation of epithelioid macrophages, which is suggestive finding of transformation to granuloma, and granuloma formation were occasionally observed. Aggregation of macrophages containing particles was observed in the pulmonary lymph nodes at 3 months after instillation. Prolonged inflammatory foci (granuloma) and presence of ASD particles in pulmonary lymph nodes would have a chance to induce immunological modulation leading to adverse health effects.

This study obtained following two conclusions.

1. Instilled nanoparticles may be internalized in the ABB by caveolae-mediatd endocytosis.
2. Instilled ASD induced granuloma in lungs and aggregation of particle containing macrophages in pulmonary lymph nodes at 3 month after exposure; these changes may have effects on immune modulation.

The information from this study may be useful in predicting the toxicity of SPM *in vivo*.