Expression of Peroxiredoxins and Pulmonary Surfactant Protein A Induced by Silica in Rat Lung Tissue*

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Silicosis is one of the most serious occupational diseases in China and dates back to centuries ago. In this study, we successfully established a rat model of silicosis by intratracheal silica injection for 28 days and determined hydroxyproline levels to evaluate collagen metabolism in lung homogenates. Oxidative stress status was evaluated by detecting catalase and glutathione peroxidase activities. Expression levels of peroxiredoxins (Prx I and Prx VI) were detected by Western blotting. Pulmonary surfactant protein A (SP-A) levels in rat serum and lung tissue were analyzed by ELISA, and SP-A and Prx expression levels in lung tissues were detected by immunohistochemistry. The results suggest that Prx proteins may be involved in pulmonary fibrosis induced by silica. Downregulation of SP-A expression caused due to silica is an important factor in the occurrence and development of silicosis.

Silicosis is a systemic disease linked to pulmonary fibrosis caused by inhalation of respirable crystalline silica (<10 μm in size) in long-term production processes. It can cause serious damage to human health, particularly in workforce personnel, when materials containing crystalline silica are reduced to dust or when fine particles are disturbed. Inhaling crystalline silica dust can lead to silicosis, bronchitis, or cancer[1]. Silicosis involves a series of biological reactions, starting with silica entry into the lung tissue. Currently, pathogenesis of silicosis includes a range of possibilities, such as involvement of free radicals, lipid peroxidation, lysosome cytokines, immune and network systems, and cell apoptosis[1]. The primary observable pathological process of silicosis fibrosis is early diffuse alveolitis inflammation, followed by pathological hyperplasia of fibroblasts and extracellular matrix collagen accumulation, which replaces the normal lung tissue structure[2]. Although the pathogenesis of silicosis has been widely reported[3], key cellular and molecular mechanisms related to silica-induced pulmonary inflammation and fibrosis formation remain unknown. The purpose of this study was to provide a brief overview of the consequences of silica exposure and discuss how a knowledge of the identified mechanisms and biological markers may contribute to an understanding of silica-induced pulmonary fibrosis.

Male rats were purchased from the Department of Experimental Animals, North China University of Science and Technology, Hebei Province, China (certificate number: SYXK-Ji 2010-0038). All animal experiments were approved by the Beijing Weitong Lihua Experimental Animal Co. Ltd (animal certificate number: SCXK-Jing 2006-0009). SiO$_2$ (<5 μm in size, ≥99%, Sigma). A total of 64 rats were adaptively fed for 1 week. Food and water were provided ad libitum. After 1 week, the rats were randomly divided into two experimental groups, with 32 rats per group. Rats in the control group were injected with 1 mL sterile saline in the trachea. Rats in the silica dust groups were exposed for different time periods (7, 14, 21, and 28 days) following a single injection of 50 mg/mL silica suspension in the trachea. After 7, 14, 21, and 28 days of the experimental time periods, rats in each group were sacrificed. Serum was separated, collected in a centrifuge tube, and stored at -80 °C. The lungs, trachea, and bronchi were removed, rinsed with saline, and weighed after drying on a filter paper to record gross pathological changes. Hematoxylin and eosin (H&E) staining and Masson’s trichrome staining were used to detect histological changes, and the degree of pulmonary fibrosis was observed.

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under a light microscope.

Data were analyzed using SPSS 13.0 for Windows. Data are presented as mean±standard deviation (SD). Samples were compared by single factor analysis of variance. Data were consistent with normal distributions. The F test was also used. Comparison of the measurement index in different dose groups and time points was performed using analysis of variance. P<0.05 was considered as statistically significant.

After silica treatment, the lung coefficients at each time point in the SiO}_2 groups were significantly higher than that in the control group (P<0.01), with a maximum lung coefficient recorded on the 28th day. Our results suggest the possibility of silicon accumulation in the lung tissue, causing early local inflammation, inflammatory cell infiltration, and ultimately pulmonary tissue edema due to formation of collagen fibers, resulting in increased weight of the lung parenchyma. Pathological findings in this study were consistent with the cytological examination. In the early stage of infection, the number of neutrophils and macrophages increased, while lymphocytes were primarily observed in the late stage.

H&E staining showed that cellular nodules were primarily formed upon exposure to silica dust after 7 days and were composed of neutrophils, macrophages, and lymphocytes. Over time, collagen formation and nodular fibrosis occurred, and the number of inflammatory cells in the lesion area decreased such that fibrous nodules were apparent after 28 days. Masson’s trichrome staining showed that the collagen fibers were red, the nuclei were gray black or gray blue, and the muscle and cytoplasm were green, exhibiting changes in fibrosis, blood vessels, and overall lung structure. Larger red areas indicated a greater degree of fibrosis. These results show that a rat silicosis model was successfully established along with development of pulmonary fibrosis over time.

Study on oxygen free radical damage and mechanisms of action regarding development of pulmonary fibrosis have been conducted\[^{[4]}\]. Lipid peroxidation damage induced by reactive oxygen species (ROS) related to free SiO}_2 dust plays an important role in the occurrence and development of silicosis and tumors\[^{[5]}\]. The results of the present study confirm that activities of the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GSH-Px) in rat lung tissue were significantly decreased after SiO}_2 dust exposure, suggesting that the antioxidant system was damaged while fighting against ROS and that the antioxidant capacity of the body is seriously reduced, thereby affecting the balance between ROS generation and removal. Silica dust exposure likely disrupted oxidation and antioxidation processes in the body, causing increased production of ROS. Free radicals then accumulate, resulting in consumption of a large number of corresponding antioxidant enzymes, such as CAT and GSH-Px, which primarily function to remove accumulated H}_2O}_2, resulting in a downward trend in CAT and GSH-Px activity\[^{[5]}\]. Collagen deposition also plays an important role in the development of pulmonary fibrosis\[^{[6]}\]. Hydroxyproline (HYP) content can reflect collagen metabolism in lung tissue and is an important index of specificity and relevance in detecting early pulmonary fibrosis. HYP levels in rat lung tissue increased significantly over time, indicating that collagen synthesis occurred more than decomposition and that composition of the extracellular matrix was upregulated. Changes in serum levels of silicon in rats exposed to silica were observed on the seventh day after exposure to silica dust, which was earlier than those observed in levels of HYP and GSH-Px. These results indicate the significance of early diagnosis of silicosis by determining the silica levels in serum.

Prx proteins are newly discovered peroxidases that play an important role in clearing ROS from the body. Prx I is one of six Prx types found in human cells and is primarily located in the cytoplasm. It is an inducible protein that is significantly expressed at higher rates during oxidative stress and in some malignant tumor cells. Prx VI occurs in the cytoplasm and can be detected in several tissues, with the highest expression in the lungs. This study evaluated Prx I and VI protein expression in the rat lung tissue following silica exposure using two methods. Western blotting (Figure 1) and immunohistochemical (Figure 2) results showed that increased exposure times led to gradual increases in Prx I and VI protein expression, with the highest levels on the 28th day following exposure. Prx proteins may be involved in pulmonary fibrosis induced by silica. Immunohistochemical results showed that Prx I protein expression in the rat lung tissue at 14, 21, and 28 days was significantly higher than that in the control group (Figure 2A), which also confirmed that Prx I stimulated the proliferation of...
epithelial cells. The pathogenesis of silicosis is related to oxidative stress caused by Prx I protein because Prx I protein has a dual function of providing antioxidant activity and stimulating epithelial cell proliferation\[^7\]. Increased Prx I expression may be related to formation of lung tissue inflammation and fibrosis induced by silica. Specifically, oxidative stress in rats induced by SiO\(_2\) dust leads to an increase in Prx I protein reactivity in order to resist oxidative stress. Therefore, the formation of rat pulmonary fibrosis induced by silica is related to increased Prx I expression in the lung tissue of rats. This mechanism will need further evaluation.

Surfactant protein A (SP-A) plays a very important role in the natural immune defense in the lungs. It can interact with several respiratory pathogens, regulate the response of white blood cells to pathogenic microorganisms, and participate in the regulation of lung immunity and inflammation\[^8\]. SP-A is expressed at high concentrations in the lungs, with very low levels being detected in other tissues. Therefore, SP-A can be used as a biological marker to detect abnormalities in lung tissues. The results of this study showed that SP-A levels in the serum of the silica-exposed groups at 14, 21, and 28 days were significantly higher than those in the control group (\(P<0.05\)) (Figure 3B), whereas SP-A levels in the lung tissues of rats exposed to silica at 21 and 28 days were significantly lower than those in the control group (\(P<0.01\)) (Figure 3A). SP-A levels in the serum may be closely related to the degree of lung injury, that is, more severe lung injuries lead to higher SP-A serum concentrations. In healthy individuals, the alveolar-capillary barrier is intact and prevents SP-A from entering the circulation, leading to detection of very small amounts of serum SP-A. Based on the results of the present study, we believe that impairments to the alveolar-capillary barrier lead to an increase in vascular permeability and subsequent

Figure 1. Effect of Prx I and Prx VI on silicosis in lung tissue. Each bar represents mean±SD (n=8); * compared with each control group, \(P<0.05\).

Figure 2. Distribution and expression of Prx I in rat lung tissue (× 200). Each bar represents mean±SD (n=8); * compared with each control group, \(P<0.01\); # compared with 7-day silica-exposed group, \(P<0.01\).
leakage of SP-A from the lungs into the circulation, resulting in increased SP-A levels in the serum. When diseases cause lung injury, SP-A can access the blood circulation via injury to the alveolar-capillary membrane barrier and become serum biomarkers of lung injury[9]. SP-A immunohistochemical staining results showed that more brown-yellow particles were found in rat alveolar type II epithelial cells and in some macrophages in the silica-exposed group at 7 days. The brown-yellow particles in the rat groups exposed to silica for 28, 21, and 14 days were significantly decreased compared to those in the 7-day treatment and control groups (Figure 3C), which could explain the significant decrease in SP-A expression in the lung tissue. We propose that silica produces cytotoxic effects in the rat lung tissue and damages alveolar type II epithelial cells and that SP-A is a specific biological indicator of lung injury. Longer exposure times lead to more severe lung injury and a significant decrease in SP-A expression, which may be related to severe damage to alveolar type II epithelial cells and decreased capacity to synthesize and secrete SP-A. Lung injury is caused by alveolar type II epithelial cell swelling, shedding, invasion of a large number of inflammatory cells, and release of a large number of oxygen free radicals, cytokines, and proteolytic enzymes. As lung damage increases, SP-A content in tissues is reduced, as reported earlier[10]. SP-A can also be reduced by injury to the alveolar-capillary membrane barrier to enter the blood circulation[9]. Therefore, we speculate that an important factor in the occurrence and development of silicosis is downregulated expression or functional deletion of SP-A caused by silica dust exposure.

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