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Exposure Time of Silica Dust and the Incidence of Oxidative Stress, Inflammation, and Fibrosis in Rat Lungs

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Abstract

BACKGROUND: Until now, exposure to silica dust is still a health problem worldwide. Silica exposure in the lungs will cause pulmonary fibrosis which is initiated by inflammation. However, the results of several studies regarding the duration of inflammation and fibrosis are still inconsistent. There was a role of oxidative stress in silicosis, but there were also inconsistencies in terms of when oxidative stress occurs in silica exposure.

AIM: This study aimed to study the toxic effects of silica dust exposure by looking at the picture of inflammation and fibrosis and malondialdehyde (MDA) levels in lung tissue during the observation period of 7 days, 14 days, 21 days, and 28 days.

METHODS: This study used a randomized post-test only control group design. The research sample was male Wistar rat (*Rattus norvegicus*), aged 6–10 weeks, body weight 150–200 g (divided into 5 groups: Control group, day 7 group, group day 14, group day 21, and group day 28). We administered silica suspension through intratracheal injection of 30 mg/rat on 0.5 mL of volume. Examination of MDA level was using the ELISA technique; histopathological examination of the liver used hematoxylin-eosin (HE) staining to determine inflammation and fibrosis. Statistical test using one-way ANOVA or Kruskal–Wallis followed by *post hoc* test.

RESULTS: The results of our study found that intratracheal silica exposure increased MDA levels on the 7th day, increased the accumulation of collagen from the 14th day, and increased the pulmonary inflammation score on the 14th day ($p < 0.05$).

CONCLUSIONS: It was concluded that silica exposure caused significant oxidative stress on day 7 as well as significant inflammation and pulmonary fibrosis on day 14.

Introduction

Silica is a chemical compound of silicon dioxide (SiO_2) which occurs mostly in crystalline form or in amorphous form in lesser amounts. These compounds are widely contained in rock, sand, and soil. Crystalline silica is also produced in several industrial processes such as mining for gold, iron, tin, granite, sand, slate, metal foundries, cement factories, ceramics, and glass [1].

Until now, exposure to silica dust is still a health problem worldwide. It was estimated that in Asia, more than 23 million people in China and 10 million people in India were exposed to silica dust in their work environment [2].

Silica exposure in the lungs will cause fibrosis which is initiated by inflammation. Pulmonary fibrosis caused by silica is commonly referred to as silicosis. Silicosis is a fibronodular lung disease caused by the inhalation of dust containing crystalline silica (alpha-quartz or silicon dioxide) into the respiratory tract characterized by the presence of alveolar proteinosis and diffuse fibrosis, causing a progressive impairment of restrictive lung function [2].

The histopathological differences of lung due to silica exposure compared to other exposures were mild-to-moderate inflammatory infiltration, formation of fibrotic foci (nodule-like fibrotic foci), and accumulation of collagen fibers within fibrotic foci. Meanwhile, on exposure to chemicals (bleomycin and paraquat), the inflammatory infiltration is severe (related to the cytotoxic properties of bleomycin and paraquat), diffuse collagen fiber deposition without the formation of obvious fibrotic foci [1].

A study conducted on the tin mining industry in China showed that there were 1015 (33.7%) workers with silicosis [2]. A study conducted on the gold mining industry in South Africa showed that 18% of black workers had silicosis in 2007 [3].

The occurrence of inflammation at the beginning of the pathogenesis of pulmonary fibrosis due to silica is not only visible on the histopathological picture of the lung but it is also characterized by an increase in inflammatory

markers, especially pro-inflammatory cytokines. Some *in vivo* studies had shown that silica exposure caused an increase in IL-1β, TNF-α, IL-6, and NF-κB level [4], [5], [6].

However, the results of several studies regarding the duration of inflammation and fibrosis were still inconsistent. The study by Zhang *et al*. [4] stated that inflammation peaked on day 7 while fibrosis occurred significantly on day 14. Inflammatory parameters of TNF-α in serum and bronchoalveolar lavage fluid (BALF) were reported to increase on day 7 and then decrease on day 14 after silica exposure [4].

These results were slightly different from the study conducted by Huang *et al*. [5]. The levels of the inflammatory parameter TNF-α in plasma were reported to increase after silica exposure over time (from 1 month to 5 months of observation), but the levels of IL-1β and IL-6 did not show a significant increase. In bronchoalveolar lavage fluid (BALF), all pro-inflammatory cytokines showed a significant increase over time [5].

Several studies had also shown the role of oxidative stress in silicosis. Research in China showed a decrease in superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (T-AOC); and increase in nitric oxide (NO), nitric oxide synthase (NOS), and malondialdehyde (MDA) after silica exposure [4], [5].

As with inflammatory and fibrotic processes, there were also inconsistencies in terms of the duration of oxidative stress on silica exposure. Research by Zhang *et al*. [4] showed that the expression of oxidative stress parameters (i.e., inducible nitric oxide synthase [iNOS]) in lung tissue on the 7th and 14th days was relatively the same. In contrast to the results of the study, Huang *et al*. [5] found an increase in reactive oxygen species (ROS) in months 1–3 and a decrease in months 4–5 after silica exposure. MDA levels were found to increase with silica exposure and this increase occurred over time. The longer the observation time, the higher the MDA level. In contrast, the levels of total antioxidants, SOD, catalase, and GPX decreased with time on silica exposure [5].

Given the inconsistence results of the previous studies regarding the time period for inflammation, oxidative stress, and fibrosis, it is deemed important to conduct research on this matter. This study aimed to study the toxic effects of silica dust exposure by looking at the features of inflammation and fibrosis as well as MDA levels in lung tissue during the observation period of 7 days, 14 days, and 21 days.

Methods

Study design

This study was an experimental study with randomized post-test only control group design. The study was conducted at Integrated Biomedical Laboratory of Medical Faculty, Udayana University, Bali. The study protocol had been approved by Ethics Committee of Medical Faculty, Udayana University, Bali, Indonesia.

Samples

Our study included 30 rats (*Rattus norvegicus*) strain Wistar, male, age 6–10 weeks, weight 150–200 g. The minimum number of samples was 28 samples which divided into four groups:

1. Control group (only received distilled water);
2. Group I (received silica suspension through intratracheal injection and observed for 7 days);
3. Group II (received silica suspension though intratracheal injection and observed for 14 days);
4. Group III (received silica suspension through intratracheal injection and observed for 21 days);

Administration of silica

Crystalline silica was prepared in suspension. Silica suspension was prepared by diluting 30 mg of crystalline silica in 0.5 mL NaCl 0.9%. Silica suspension was administered through intratracheal injection.

Assessment of malondialdehyde level

The malondialdehyde (MDA) level in the lung was measured using thiobarbituric acid reactive substances assay. The MDA level was expressed in μM.

Histopathology examination

The histopathology examination of the lung was performed with hematoxylin-eosin staining. The histopathology feature evaluated including inflammation score and collagen density. The inflammation score of the lung is expressed as Table 1.

Table 1: Inflammation score of the lung

Score	Observation result
0	No inflammation
1	Local inflammation
2	The majority of bronchus and veins were surrounded by thin layer of inflammatory cells (1–5 cells)
3	The majority of bronchus and veins were surrounded by thin layer of inflammatory cells (more than 5 cells)
4	Complete inflammation in all bronchus and veins

Data analysis

Statistical analysis was performed using one-way ANOVA and *post hoc* test. The $p < 0.05$ was

considered to be statistically significant.

Results

MDA level in the lung

The study result revealed that the MDA level in rat lung after the administration of silica suspension through intratracheal injection reached highest level in 7 days and then decreased in 14 and 21 days ($p < 0.001$) (Table 2). *Post hoc* analysis also showed similar result.

Table 2: The MDA level on rat lung after the administration of silica suspension

Group	MDA (μM)	p
I (control)	21.7 ± 0.33	<0.001
II (silica 7 days)	27.8 ± 0.49	
III (silica 14 days)	10.7 ± 0.16	
IV (silica 21 days)	10.3 ± 0.41	

Histopathology examination showed lung inflammation on rats after silica exposure. Inflammatory score of the rats lung after silica exposure is shown in Table 3. The study reported significant sign of inflammation in the lung on 14 days after silica exposure.

Table 3: Inflammation score of the rat lung after silica exposure

Group	Inflammatory score
I (control)	0
II (day 7 post-silica exposure)	1
III (day 14 post-silica exposure)	3
IV (day 21 post-silica exposure)	2

The percentage of collagen density commonly indicated the degree of fibrosis in the lung. In lung, fibrosis generally showed collagen accumulation in lung histopathology. The collagen density in rats lung is shown in Table 4.

Table 4: Collagen density in rat lung after the administration of silica suspension

Group	Collagen density (%)	p
I (control)	25.5 ± 0.24	<0.001
II (silica 7 days)	18.7 ± 0.29	
III (silica 14 days)	35.1 ± 0.49	
IV (silica 21 days)	29.0 ± 0.33	

Our study revealed significant increase of collagen density in lung tissue 14 days and 21 days after silica exposure compared to control group ($p < 0.000$). *Post hoc* analysis also reported similar result ($p < 0.000$).

Discussion

Silica dust exposure in the lungs has been proved to cause pulmonary inflammation and fibrosis which is commonly referred to as silicosis. Inhalation of dust containing crystalline silica into the respiratory tract would lead to progressive impairment of restrictive lung function. This would manifest as decreased of expansion capacity, vital capacity, and diffusion capacity of the lungs [2].

The types of pulmonary fibrosis caused by prolong exposure to fibrogenic agents such as silica dust commonly are interstitial pulmonary fibrosis. The process of pulmonary fibrosis involves inflammation and disruption of normal tissue architecture, which is then followed by a healing process with the accumulation of mesenchymal cells and excessive production of extracellular matrix. Macrophages and fibroblasts are target cells involved in the occurrence of pulmonary fibrosis. As a result of inhalation of silica particles, alveolitis is initiated by activating inflammatory cells which, in turn, induces interstitial pulmonary fibrosis. Oxidative stress can trigger inflammation that will continue with fibrosis. Inflammation occurs through the activation of NF-κB [1], [2], [7].

There are several differences on histopathological features of lung due to silica exposure compared to other fibrogenic agent exposures (bleomycin or paraquat). These are including the severity of inflammation, the distribution of collagen, and the formation of fibrotic foci. On silica exposure, the inflammation commonly is mild to moderate, accompanied by the formation of fibrotic foci (nodule-like fibrotic foci), and accumulation of collagen fibers within fibrotic foci; whereas on exposure to other chemicals (bleomycin and paraquat), the inflammatory infiltration is commonly severe, accompanied by diffuse collagen fiber deposition without the formation of obvious fibrotic foci [1].

ROS in the lungs can be derived from electron transport in mitochondria, myeloperoxidase, xanthine oxidase, and NADPH oxidase (NOX). The most concern is NOX, namely, NO4. NOX4 is an isoform of NOX which is increased by TGF-β. The latest mechanism is that ROS can activate latent TGF-β and TGF-β can also increase ROS production in human lung fibroblasts. TGF-β lowers GSH, SOD, CAT, and GCS levels and increases H_2O_2 [8], [9].

Our results showed that there was a significant increase in MDA levels on day 7 in lung tissue after exposure to silica at a dose of 30 mg compared to controls. Furthermore, there was a decrease in MDA levels as a parameter of oxidative stress on days 14 and 21.

Our findings were similar to other studies regarding the occurrence of oxidative stress on silica exposure. Research in China showed a decrease in superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (T-AOC); and increases in nitric oxide (NO), nitric oxide synthase (NOS), and malondialdehyde (MDA) after silica exposure [4], [5], [6].

However, the time period for the occurrence of oxidative stress after silica exposure were varied in other studies. Research by Zhang *et al*. (2018) showed

that the expression of oxidative stress parameters (i.e., iNOS) in lung tissue peaked at the 7th and 14th days after silica exposure [4]. This was in contrast to the results of the study by Huang *et al*. [5]. Huang *et al*. found an increase in ROS in months 1–3 and a decrease in months 4–5 after silica exposure. MDA levels were found to increase after silica exposure and this increase occurred over time. The longer the observation time, the higher the MDA level. In contrast, the levels of total antioxidants, SOD, catalase, and GPX decreased with time after silica exposure [5].

It is well known that oxidative stress can stimulate inflammation. The occurrence of inflammation at the beginning of the pathogenesis of pulmonary fibrosis due to silica is not only visible on the histopathological feature of the lung but it is also characterized by an increase in inflammatory markers, especially pro-inflammatory cytokines. *In vivo* studies have shown that silica exposure causes an increase in IL-1β, TNF-α, IL-6, and NF-κB [4], [5], [6], [10], [11], [12].

Our study reported significant sign of inflammation in the lung on 14 days after silica exposure. Slightly different from our study, the study by Zhang *et al*. [4] stated that inflammation peaked on day 7 while fibrosis occurred significantly on day 14. Inflammatory parameters of TNF-α in serum and BALF were reported to increase on day 7 and then decrease on day 14 after silica exposure [4].

These results were also slightly different from the study conducted by Huang *et al*. [5]. The levels of the inflammatory parameter TNF-α in plasma were reported to increase after silica exposure over time (from 1 month to 5 months of observation), but the levels of IL-1β and IL-6 did not show a significant increase. In bronchoalveolar lavage fluid (BALF), all pro-inflammatory cytokines showed a significant increase over time [5].

Our study revealed significant increase of collagen density in lung tissue 14 days and 21 days after silica exposure compared to the control group. The previous *in vivo* studies have demonstrated a role for TGF-β in lung fibrosis induced by silica exposure. Animal studies have shown that TGF-β levels are increased in pulmonary fibrosis due to silica exposure [6], [10], [13], [14], [15], [16], [17].

TGF-β is the main profibrotic growth factor that can stimulate fibroblast extracellular matrix (ECM) production, myofibroblast differentiation, resistance to apoptosis, and ROS production; induces epithelial cell apoptosis; and stimulates epithelial-mesenchymal transition (EMT). TGF-β is often referred to as a multifunctional cytokine because it regulates the proliferation, differentiation, and production of ECM. In fibrosis, there is an increase in TGF-β [8], [18]. TGF-β1 is the most commonly found isoform and is expressed in almost all cell types in an inactive complex form. To bind to its receptor on the cell surface, TGF-β must be converted to its active form. Molecules that can convert TGF-β into active are metalloproteinases (MMP-2 and MMP-9). In conditions of tissue injury and inflammation, TGF-β triggers proliferation by activating other growth factors such as connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), or epidermal growth factor (EGF) [19], [20], [21], [22], [23].

The TGF-β signaling pathway is divided into a canonical or Smad-dependent pathway and a non-canonical or Smad-independent pathway. The canonical TGF-β pathway is initiated by the activation of TGFRII by the TGF-β2 ligand which, in turn, forms a heteromeric complex with TGFRI (also known as activin receptor-like kinase; ALK) and causes recruitment and phosphorylation (activation) of TGFRI receptors. Dimerization of the two receptors can also occur when there is binding of TGF-β1 to TGFRI. Furthermore, TGFRI activation phosphorylates Smad2 and Smad3 at the C-terminal serine residue, resulting in the formation of heterodimeric and trimeric complexes with the common Smad (co-SMAD), namely, Smad4. This causes the translocation of the complex to the nucleus and binds to the Smad-binding element (SBE) on the promoter of the target gene, acting as a transcription factor in regulating the expression of TGF-β target genes (such as α-sma, collagen). Apart from the Smad protein, TGF-β can also activate other signaling systems, such as Erk1/2, JNK, TAK1, PI3K, and p38 MAPK. This signaling system plays a role in various cell activities, such as cell growth and differentiation, apoptosis, cell motility, extracellular matrix production, angiogenesis, immune response, and epithelial-mesenchymal cell transition [20], [21], [22], [23].

This study clarified more specifically about the toxic effects of silica on the lungs at various time periods by looking at the signs of inflammation, oxidative stress, and fibrosis in lung tissue. However, the parameters observed in this study were limited to pulmonary histopathological features (related to inflammation and fibrosis) and pulmonary MDA levels. This study did not evaluate more specific markers of inflammation, oxidative stress, or fibrosis (at the molecular level) such as TNF-α, TGF-β, IL-1β, IL-6, SOD, iNOS, and GPX.

Conclusions

The highest level of MDA in the lung was observed after 7 days of silica exposure, whereas significant inflammation and fibrosis of the lung were observed after 14 days of silica exposure.

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