# A STUDY ON CHANGE OF TYPE I AND III COLLAGEN DURING FIBROSIS INDUCED BY SILICA AND WELDING FUME DUST

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## ABSTRACT

ELISA method was used to study the quantity and distribution of type I and III collagen in lungs of rats induced by silica and welding fume dust. The ratio of I/III collagen was obtained and tested for evaluation of the degree of fibrosis. On the 10th day after instillation of silica, I/III collagen ratio was lower than normal. After 20 days, it increased significantly and stayed at constant level since then. Similar type change of collagen was also observed from histological specimens. Increase of Type III collagen appeared in the early stage of fibrosis and Type I collagen increased more rapidly in the later stage.

In lungs of rats instilled with welding fume dust, Type III collagen increased predominantly until 180 days after instillation, while significant increase of Type I collagen was observed not until after 180 days. It induced a slower and milder fibrosis in the lung. Ratio of Type I/III collagen contents can be used to evaluate the degree of fibrosis.

## INTRODUCTION

The main characteristic of lung fibrosis is massive increase of interstitial collagen in the lung. The study of type change of lung collagen may be helpful to understand the process of fibrosis. In this study, ELISA method was applied to determine the contents and distribution of Type I and III collagen. The ratio of these two kinds of collagen was tested for evaluation of degree of fibrosis induced by silica and welding fume dust.

## MATERIALS AND METHODS

1. Rats

   Female Wistar rats were used. Body weights were about 200 g.

2. Dusts

   a. Quartz 95% of quartz with particle sizes smaller than 5 μm. Free silica content was about 97%. 50 mg of quartz were instilled to each rat intratracheally.
   b. Welding fume dust (Ji-507) 95% of the welding fume dust particles were smaller than 5 μm. Dosage of dust for each rat was 50 mg.

3. Preparation of Type I and III collagen and their antibodies. The procedures were the same as described in Reference 1.

4. Method for determination of Type I and III collagen content in the lung. Rat lungs were dipped in acetone for 2 days, then dried and pulverized. To 25 mg of the dried lung powder, 5 ml of 0.5 mol acetic acid containing 5 ml of pepsin solution (2 mg/ml) were added and collagens were extracted for 24 hrs. The supernatants obtained after centrifugation were used for determination of Type I or III collagen contents with ELISA method.

5. Histological study of distribution of Type I and III collagen in the lung. Lung tissue slices were soaked in 1% peroxidate solution to inhibit the intrinsic peroxidase activity. After rinsing with saline, they were covered with collagen antiserum (Type I or III) and incubated at 37°C for 1 hr. Rinsed with phosphate buffered saline (PBS). The slices were then covered with hydrogen peroxidase labelled IgG at 37°C for 30 min. Washed with PBS again. The slices were dried, dehydrated and fixed and then were observed under the microscope to study the distribution of Type I and III collagen and their relative contents were determined by microscopic specrophotometric analysis.

## RESULTS

1. Changes of Type I and III collagen in silicotic rat lung.

   The contents of Type I and III collagen in silicotic rat lung were both increased continuously as the time prolonged after dusting. At 10 days after dusting the ratio was about 2 and kept at constant level until 90 days after dusting (Figure 1, Table I). Histological study of collagen fibers in the slices showed that after dusting, the alveolar septa and lung interstitial were all expanded and accumulated with Type I and III collagen. At 10 days after dusting with silica, there was mainly Type III collagen appearing in the lung, while at 20 days, there was mainly type I collagen present in the lung (Figure 1). This indicated that Type III collagen increased predominantly at early stage of silicosis and Type I collagen increased predominantly at later stage of silicosis.

Table I

Change of Type I and III Collagen Contents in Lungs of Silicotic Rats

| Group | Days after dusting | Collagen content (mg/g protein) | | I/III Ratio |
|---|---|---|---|---|
| | | Type I | TypeIII | |
| Normal | -- (6) | 38.9 + 6.2 | 28.2 + 4.6 | 1.38 |
| Silicotic | 10 (6) | 48.4 + 10.3 | 54.6 + 2.7** | 0.89 |
| | 20 (6) | 172.6 + 66.7** | 67.6 + 22.5* | 2.55 |
| | 30 (6) | 239.5 + 109.7* | 113.8 + 26.8** | 2.10 |
| | 60 (6) | 291.0 + 92.1** | 145.1 + 54.3** | 2.10 |
| | 90 (6) | 353.8 + 111.4** | 177.6 + 29.4** | 1.99 |

* $P < 0.01$, compared with the normal control

** $P < 0.001$, compared with the normal control

( ) Number of rats indicated in the parenthesis

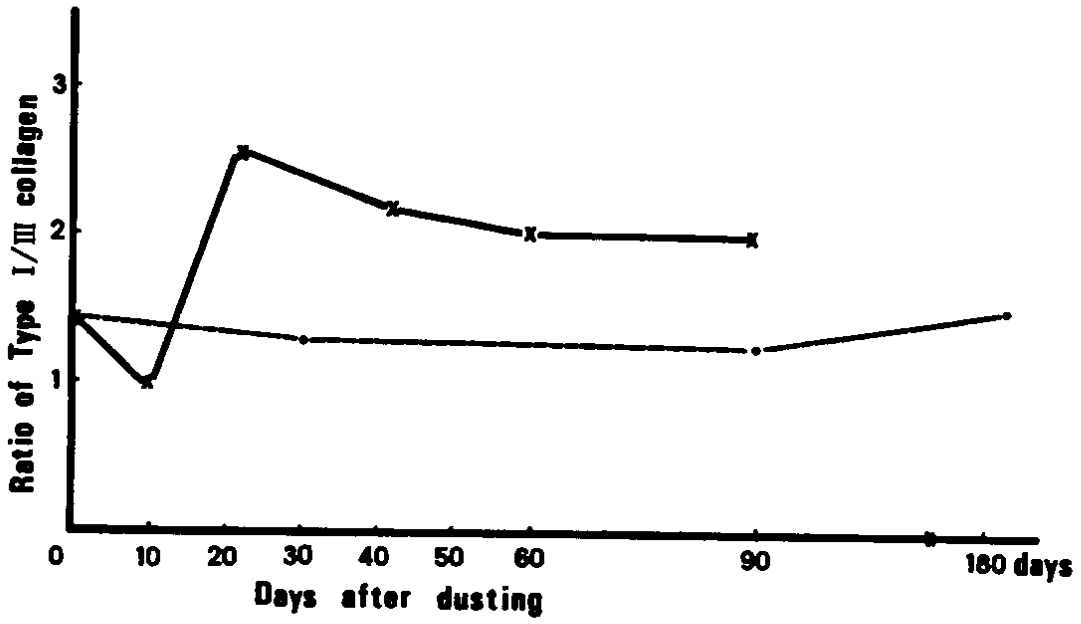


Figure 1. Ratio of Type I/III collagen in rat lung after dusting.

——— Silica

– – – – – Welding fume dust

2. Change of Type I and III collagen in lungs of rats instilled with welding fume dust.

   At 30 days after installation with welding fume dust, the content of Type III collagen increased significantly, but significant increase of Type I collagen was not observed until 180 days after dusting (Table II). The ratios of I/III collagen in the lung decreased gradually within 90 days and were raised to nearly normal level at 180 days (Figure 1). The results showed that this kind of welding fume dust induced a slower and milder lung fibrosis as compared to silicosis. Histological observation confirmed this results (Figure 3).

## DISCUSSION

The increase of lung collagen was usually expressed by increase of hydroxyproline. In this paper, we used ELISA method to determine both Type I and Type III collagen. The privilege of this method is that collagen contents and change of type of collagen in the fibrotic process can be determined directly. Through comparison of Type I/III collagen ratio, the fibrogenic ability of various dusts can be demonstrated. By ELISA staining method the distribution of Type I or III collagen in the lung can be observed, while all other methods do not differentiate the collage types.

Table II

Change of Type I and III Collagen Contents in Rats Lung Instilled with Welding Fume Dust

| Group | Days after dusting | Collagen content (μg/g protein) | | I/III Ratio |
|---|---|---|---|---|
| | | Type I | Type III | |
| Normal | -- (6) | 38.9 + 6.2 | 28.1 + 4.63 | 1.38 |
| Silicotic | 30 (6) | 48.1 + 6.6 | 38.4 + 5.4 ** | 1.25 |
| | 90 (6) | 40.0 + 2.7 | 35.2 + 2.6 * | 1.13 |
| | 180 (6) | 69.0 + 21.5 * | 48.9 + 8.2 ** | 1.41 |

* $P < 0.05$, compared with the normal control

** $P < 0.01$, compared with the normal control

( ) Number of rats indicated in the parenthesis

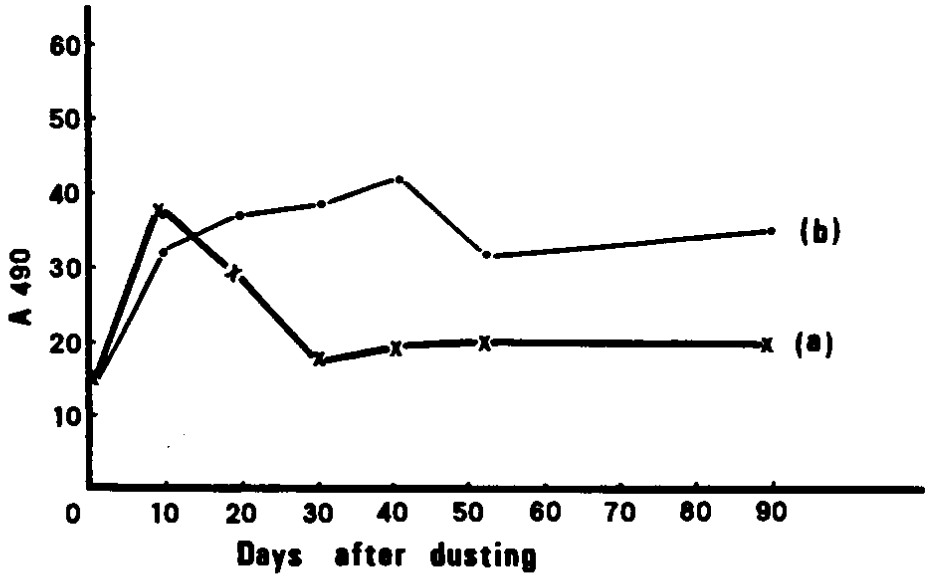


Figure 2. Microscopic spectrophotometric analysis of silicotic lung slices stained with ELISA method.
a. Type III collagen, b. Type I collagen

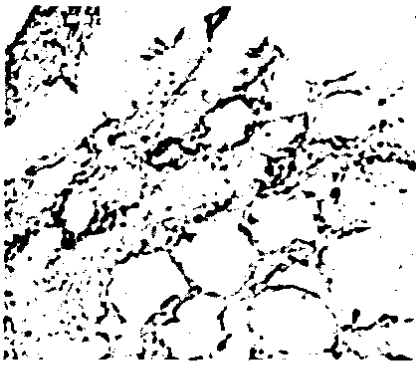

Figure 3. ELISA staining of normal lung (collagen Type I).

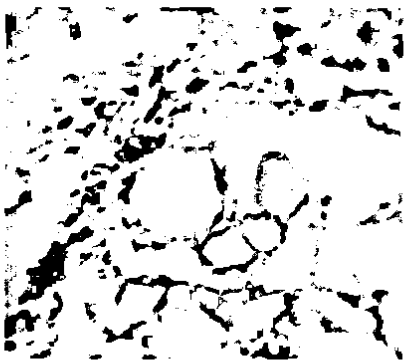

Figure 4. ELISA staining of normal lung (collagen Type III).

Figure 5. ELISA staining of $SiO_2$ dusting lung (1 month, collagen Type I).

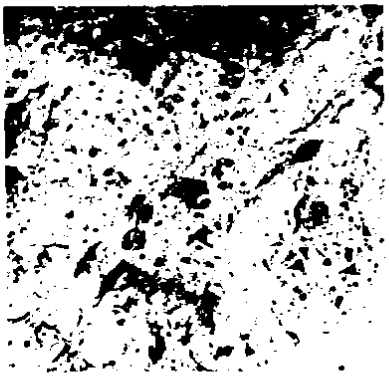

Figure 7. Welding fume dust lung specimen (ELISA staining, 3 month, collagen Type I).

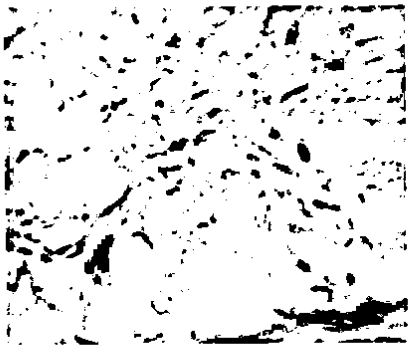

Figure 6. ELISA staining of $SiO_2$ dusting lung (1 month, collagen Type III).

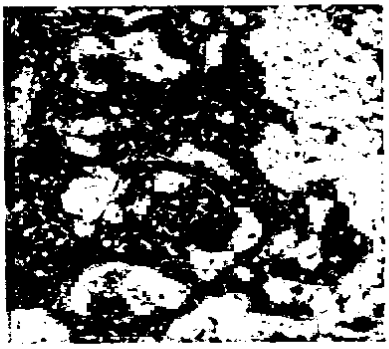

Figure 8. Welding fume dust lung specimen (ELISA staining, 3 month, collagen Type III).

Reiser[2] in his study reported that I/III ratio was constant during silicosis, but in our study, a sharp decrease of I/III ratio on 10th day after dusting and an obvious increase on the 20th day were observed which indicated that Type III collagen increased predominantly in the early stage of fibrosis. This fact is similar to those reported by Ganesh[3] in the study of adult respiratory distress syndrome.

Comparison of I/III ratio between rat lung dusted with silica and welding fume dust showed that the difference was significant. The ratio of I/III for welding fume dust was lower, the type change was not so obvious as those in silicotic fibrosis. It proved that I/III ratio can be used for evaluation of fibrogenicity of dusts.

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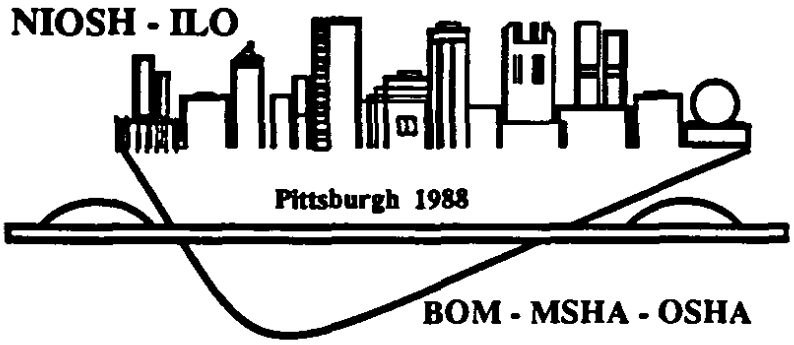


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