Silicon Carbide Induced Pneumoconiosis: A Microscopic and Biochemical Experimental Study

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Abstract: Silicon Carbide Induced Pneumoconiosis: A Microscopic and Biochemical Experimental Study: Marilena PETRAN, et al. Hospital of Occupational Medicine Cluj-Napoca—The pneumoconiogenic effects of silicon carbide were studied in an experimental model by intratracheal instillation of a dose of 50 mg silicon carbide, standardized and commercialized by Duke Scientific Corporation, in a group of 30 guinea pigs killed at 40, 70 and 100 days, respectively. Lung samples were obtained and they were fixed, included, sectioned, stained with hematoxylineosin, van Gieson and Masson’s trichromic and studied under the optical microscope. The microscopic aspects were compared with those of the controls (10 guinea pigs), intratracheally instilled with 1 ml physiological solution (NaCl 0.9%) at the beginning of the experiment and killed with the last group of guinea pigs at 100 days. The optical microscopic study showed important changes in the pulmonary structure: alveolar wall ruptures, infiltration of alveolar septa with round cells and histiocytes, as well as diffuse interstitial fibrosis which was more obvious 100 days after the instillation. Lung tissue hydroxyproline, as a marker of collagen synthesis, showed a significant increase correlated with the occurrence of fibrosis. The results of the experimental research support the pneumoconiogenic effect of silicon carbide.

(J Occup Health 1999; 41: 253–258)

Key words: Silicon carbide, Pneumoconiosis, Diffuse interstitial fibrosis, Hydroxyproline, Experiment

Classical experiments, carried out more than 50 years ago by Gardner1 refuted the fibrogenic effects of silicon carbide (SiC) on the lung. Gardner used these results as arguments against the mechanical theory of silicosis, which attributed the essential causative role in pneumoconiosis to silica hardness. According to his data, SiC, which is harder the quartz, had no fibrogenic effects. Gardner’s outstanding scientific personality hindered the initiation of other experiments for a long time. During that period, the few reports on pneumoconiosis cases based on radiological findings were the result of occupational exposure to abrasive mixtures, including aluminium oxides whose fibrogenic potential is well known.

In the last 20 yr, the increased production of artificial abrasives, among which SiC occupies the primary place, and the obtaining of new predominantly fibrous varieties have required further research on the effects of occupational exposure. The continuation of this research has also been the consequence of research programs on the aggressiveness of artificial mineral fibers, where SiC has been included. There continue to be described isolated cases: the case of a glass worker2, that of a dental technician using SiC for dental ceramics3, but epidemiological studies are also being published, concerning the causes of death among artificial abrasive manufacture workers4, as well as morphological aspects of human pneumoconiosis5–7. Radiological observations in large groups of workers exposed to SiC dust8–10 have showed, with variable frequency, the occurrence of small pneumoconiotic radiological opacities.

The relatively low number of publications on experimental pathology is surprising. The use of pure, standardized SiC varieties provides the basis for a more correct interpretation of the lesions they cause. The aim of this paper is to evaluate the morphological pulmonary response to SiC aggression, along with hydroxyproline dosing used as a biochemical marker of fibrosis.

Materials and Methods

The experiment was performed on guinea pigs. A group of 30 animals, with a mean weight of 336.5 ± 80.2...
were instilled intratracheally with standard SiC and a group of 10 animals, with a mean weight of 321.4 ± 70.3 g were instilled intratracheally with a physiological solution (NaCl 0.9%). All the animals were male. The differences in weight between the two groups at the beginning of the experiment were statistically insignificant (p>0.10). In both groups, the instillation was performed under ether narcosis. The 30 guinea pigs from the SiC treated group were instilled intratracheally with a single dose of 50 mg standardized SiC suspended in 1-mL physiological solution, commercialized by Duke Scientific Corporation (catalog no. 349) and which does not contain free crystalline silicon dioxide. We verified its absence by X-ray diffraction at the Institute of Chemistry “Raluca Ripan” of Cluj-Napoca. This variety is formed by particles between 0.7 and 7 μm in diameter, most of them being respirable, which we checked against the LASER granulometric study. The animals in the control group were treated under similar conditions with a single dose of 1-mL physiological solution. Recovery from narcosis was normal and there were no complications during the days after the instillation. The animals were maintained under laboratory conditions and were given the usual food. During the experiment, the animals were killed in groups of 10 under prolonged ether narcosis. One group was killed at 40 days, a second group at 70 days, and the third group at 100 days. Along with this latter group, the controls were also killed. At the time of killing, the weight of each animal was evaluated, including the surviving animals and the controls. After the thorax was opened, the in situ macroscopic aspects of the lungs and pleura were examined; the lungs were weighed and the lung tissue was sectioned. Fragments were collected from the base of the upper lobes and the tip of the lower lobes and they were suspended in a formol solution for the microscopic morphological examination. The fragments fixed in formol were included in paraffin, sectioned and stained with HE, van Gieson and Masson’s trichromic. The remainder of both lung tissues was prepared by the removal of bronchial airways, and introduced in special recipients stored with a thermostat at 60°C, being weighed daily until a constant weight was reached. During this phase, the content of each recipient was treated with 4-M hydrochloric acid 5M, after which it was hydrolyzed at 145°C for 3 h in hermetically closed steel tubes. From the fluid resulting after hydrolysis, hydroxyproline was administered by the Neumann-Logan method11). The hydroxyproline content was expressed in mg/100 mg dry lung tissue and was dynamically evaluated, 40, 70 and 100 days after the instillation, being compared with that found in the controls. The statistical method employed in this paper was the Student’s test.

### Results

Observations on the evolution of weight parameters have been reported on other occasions12, 13), whereas the weight of the control animals showed a slight constant increase, in the SiC treated animals a transient decrease was found after instillation, which subsequently returned to normal (Table 1). With regard to the lung weight, at the end of the experiment this was significantly lower than in the SiC treated group. There were no important macroscopic changes, except for the inconstant presence of anthracotic pigmentary areas against a paler background of the sections, and in some, pleural adherences, all these changes being exclusively noted in the animals killed 100 days after the instillation.

The aspects observed in the microscopic sections are polymorphous and they involve several structural elements. Compared to the normal aspect, observed in the control group (Fig. 1), lesions differ in relation to the time elapsed since the instillation. At 40 days, there were frequent alveolar wall ruptures with dilatation of alveolar spaces and diffusion round cell and histiocytic septal infiltration frequently resulting in nodular aspects (Fig. 2). There is a marked tendency to bronchial and arteriolar wall thickening. At 70 days, lesions are obviously more marked. The alveolar wall destruction expands, there are enlarged alveolar spaces with round cell and

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (arithmetic mean ± SD)</th>
<th>Lung weight (arithmetic mean ± SD)</th>
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<tbody>
<tr>
<td>Control*</td>
<td>349.8 ± 80.2</td>
<td>3.3 ± 0.22</td>
</tr>
<tr>
<td>SiC1 (40 days)</td>
<td>279.2 ± 118.9</td>
<td>3.7 ± 0.63</td>
</tr>
<tr>
<td>SiC2 (70 days)</td>
<td>417.7 ± 83.7</td>
<td>5.3 ± 0.97</td>
</tr>
<tr>
<td>SiC3 (100 days)</td>
<td>385.5 ± 83.5</td>
<td>4.6 ± 0.48</td>
</tr>
</tbody>
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*Unique control group killed at 100 days.
histiocytic infiltration walls, and cell granulomas are numerous, having a predominantly perivascular (Fig. 3) and peribronchial (Fig. 4) location. The arterial walls are thickened, fibroed and infiltrated with fibroblasts, and the thickened bronchial walls form a fibrous reaction. Peribronchial and perivascular fibrosis is more pronounced in the microscopic sections stained with Van Gieson Stain (Fig. 5), where the tendency to extend in the neighboring alveolar septa is frequently noted. One hundred days after the instillation, in the same section the aspects range from perivascular and peribronchial cell nodes to massive fibrosis of arteriolar and bronchial walls. Round cell and fibroblast wall infiltration is massive. Arterioles are frequently almost completely obliterated and the bronchial submucosa shows inflammatory infiltrations (Fig. 6). During this stage, the fibrotic process obviously extends to the alveolar septa from the proximity of the vessels and bronchi (Fig. 7), showing the appearance of diffuse interstitial fibrosis. No lesions similar to silicotic nodules were noted.

Hydroxyproline increased slowly but progressively during the experiment (Fig. 8). At 40 days, the increase was already significant compared to the control group. During this stage, only a diffuse septal infiltration and a granulomatous reaction were morphologically noted. At 70 days, the mean hydroxyproline value was 2.32 ± 1.61 mg/100 mg lung tissue, obviously increased compared to the control group but, due to individual variations reflected by the high standard deviation, it did not reach statistical significance. Fig. 9 shows the log normal distribution and geometric mean of hydroxyproline values. In accordance with the peribronchovascular fibrosis changes and their interstitial development, at 100 days hydroxyproline was over 3 times higher, a value which was very significant (p<0.001) compared to the control. Microscopically findings of silicon carbide instilled in the animals are shown in Fig. 10.
Fig. 6. One hundred days after SiC instillation. Massive fibrosis of arterial walls and peribronchial fibrosis with a tendency to extend to the neighbouring septa. HE × 200.

Fig. 7. One hundred days after SiC instillation. Peribronchial and perivascular fibrosis extending to the neighbouring septa. Masson’s trichromic × 200.

Fig. 8. Lung tissue hydroxyproline (mg hydroxyproline/100 mg dry lung) SiC1 = 40 days after SiC instillation, SiC2 = 70 days after SiC instillation, SiC3 = 100 days after SiC instillation.

Discussion

To date, there is no consensus concerning the effects of SiC on the lung under experimental conditions. Some researches have refuted its capacity to induce pneumoconiosis\(^{14,15}\) but there are also observations indicating that SiC causes obvious lesions\(^{16,17}\). By experimenting on sheep, Begin et al.\(^{16}\) noted the appearance of a fibrosing alveolitis and Vaughan et al.\(^{18}\) described an important granulomatous reaction. Our observations confirm both types of lesions, which appear gradually, fibrotic lesions following the formation of cell granulomas.

Since pneumoconiosis is defined by the deposition of mineral dust in the lungs and the reaction of the tissue to its presence, we consider that under the experimental conditions achieved by us, SiC induces pneumoconiosis. From a histological point of view, this does not resemble silicosis, which is characterized by fibrocellular and fibrohyaline modules. In the more advanced stages, the microscopic aspect suggests diffuse interstitial fibrosis, the lesions being very similar to those described by Hayashi and Kajita\(^{7}\) in a case of human pneumoconiosis. The same authors described perivascular and
peribronchial granulomas with parietal thickenings and a collagen reaction with hyalinization in the conglomeration regions.

Our experimental study showed that this pneumoconiosis is accompanied by an increase in hydroxyproline in the lung tissue. This change has not been reported in lung pathology following SiC aggression and may be considered a marker of the fibrosis process. This amino acid is characteristic of the connective-collagen structures.

To conclude, the effects of intratracheal instillation of a single dose of 50 mg standardized SiC induce in guinea pigs pneumoconiotic lesions of granulomatous and diffuse interstitial fibrotic appearance, which is expressed biochemically by a hydroxyproline increase in the affected lung tissue.

References