

Neoplastic lung lesions in rat after chronic exposure to crystalline silica

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Groups of 100 SPF Fischer-344 rats were exposed 6 h a day, 5 d a week for 24 months to crystalline silica ($1 \text{ mg} \cdot \text{m}^{-3}$, DQ 12 quartz) or titanium dioxide ($5 \text{ mg} \cdot \text{m}^{-3}$) or air only. The animals were kept without further exposure for an additional 1.5 months. In the group exposed to crystalline silica a significantly increased incidence of 20 primary lung tumors was observed among 19 animals. The distribution of tumor types consisted of 3 adenomas, 11 adenocarcinomas, 4 benign cystic keratinizing squamous-cell tumors, 1 adenosquamous carcinoma, and 1 squamous-cell carcinoma. There were also 13 nodular hyperplasia lesions, which were interpreted to be borderline cases of adenomas. Approximately half of the adenoid tumors and all of the nodular hyperplasia lesions were characterized by moderate central fibrosis. The principal nonneoplastic findings in the silica-exposed group were lipoproteinosis, inflammation, epithelial hyperplasia, and fibrosis. The results can be considered significant due to the increased lung tumor incidence at a relatively low exposure level.

Key terms alpha-quartz, animal, Fischer-344 rats, fibrosis, inhalation, lung tumor.

This investigation was designed as part of a more comprehensive study in which the biological effects of long-term inhalation of a special test toner material, enriched in respirable particles, was evaluated. Two materials, titanium dioxide and crystalline silicon dioxide (silica), were used as positive and negative controls for fibrogenicity. First, results of effects after quartz exposure were published as a brief communication (1). Details of the entire study have been reported separately (2, 3).

Materials and methods

The silicon dioxide (SiO_2), type DQ-12, used in the study originated from Bergbau Forschung, Essen, Germany (4). X-ray diffraction analysis indicated 87% crystallinity as α -quartz. The mass median aerodynamic diameter (MMAD) was about $1.3 \mu\text{m}$ with a geometric standard deviation of 1.8, and the respirable fraction was 74% according to criteria of the American Conference of Governmental Industrial Hygienists (ACGIH). Titanium dioxide (TiO_2), type "Bayertitan T," was obtained from Bayer AG, Krefeld, Germany. A chemical analysis showed the material was 99.5% rutile TiO_2 . The mass median aerodynamic diameter was about $1.1 \mu\text{m}$ with a geometric standard deviation of 1.6, and the respirable fraction was 78% according to the ACGIH criteria.

A dry aerosol dispersion technique was used (5). Groups of viral antibody-free specific pathogen-free Fischer-344 rats were exposed to crystalline silica ($1 \text{ mg} \cdot \text{m}^{-3}$) and titanium dioxide ($5 \text{ mg} \cdot \text{m}^{-3}$) in a two-year study. A third group inhaled filtered air and served as controls. The rats (50 male and 50 female rats per group, 8 weeks of age at the start of the study) were exposed in

horizontal flow type whole-body inhalation chambers for 6 h a day, 5 d a week for 24 months under specific pathogen-free (SPF) barrier conditions. The final sacrifices started six weeks after the end of the exposure period. The measured virology, bacteriology, and parasitology parameters were within normal limits or negative during the study (2, 3). The degree of fibrosis was graded according to Wagner (6).

Results

No treatment-related effects on life span or causes of death were observed. The median life span was 750 d after the start of exposure, corresponding to a median life span of 806 d for all the animals.

The wet-lung weight of the silica-exposed animals doubled, while the TiO_2 -treated animals had lung weights similar to those of the control group. The mean retained particle mass was 2.72 mg per lung for the TiO_2 group and 0.91 mg per lung for the silica group at the end of the exposure period (pooled data of males and females (3)). The fraction of the material retained in the lung-associated lymph nodes of the silica-exposed rats was much higher than that of the TiO_2 -exposed group at all time intervals. This finding suggests progressive and massive movement of the crystalline silica from the lung to the lung-associated lymph nodes. Significant cytological changes in the bronchoalveolar lavage fluid and substantial alveolar clearance retardation were observed (2, 3).

The principal nonepithelial findings, which increased with silica exposure, were as follows. Multifocal lipoproteinosis was seen in the silica-exposed group with and adjacent to fibrotic areas, and

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cholesterol clefts were also present. Foamy macrophages containing lipid substances were observed in 98% of the rats after silica exposure and in only 1% of the rats after TiO₂ treatment. Intra-alveolar and interstitial inflammatory cell infiltrates consisting mainly of polymorphonuclear leukocytes were seen in about 70% of the rats after silica exposure. This effect was not observed in the control and TiO₂-exposed groups. A moderate degree of lung fibrosis was observed in 92% of the silica-exposed rats by the termination of the study. The fibrosis was generally multifocal and predominantly located in the subpleural and peribronchiolar region. The lung collagen content was more than doubled. A small but statistically insignificant incidence of fibrosis was seen in the TiO₂-exposed group.

Epithelial alterations. Bronchoalveolar hyperplasia of the alveolar type, characterized by type II pneumocytes, was a rare finding in the control group and occurred in only a few cases in association with alveolar histiocytosis. In the TiO₂-exposed group, this lesion was observed in 9% of the rats. Among the silica-exposed animals, an increase in bronchoalveolar hyperplasia from a slight to a moderate multifocal degree was observed during the exposure period. In these animals, the alveolar walls close to the bronchoalveolar junction (centroacinar region) were lined with a single layer of cuboidal, basophilic cells. At the end of the exposure period, bronchoalveolar hyperplasia was also seen in subpleural areas and in areas of fibrosis and inflammation. In 95% of the silica-exposed rats, bronchoalveolar hyperplasia was also observed.

A focal to multifocal bronchoalveolar hyperplasia of the bronchiolar type, characterized by ciliated cells and Clara cells, was observed in about 80% of the silica-exposed rats. In the females this lesion was medium, whereas in the males the effect was less pronounced.

In the silica-exposed group at the time of death 13 cases of nodular bronchoalveolar hyperplasia were apparent which were interpreted as borderline cases to adenomas. The occurrence of nodular hyperplasia was about equally distributed between both genders. These well-circumscribed lesions had a size of up to 3 mm in diameter and were composed of a single layer of cuboidal to columnar basophilic cells. The alveolar lumina were filled with polymorphonuclear neutrophilic leukocytes and cell debris, and the interstitium was also severely infiltrated by polymorphonuclear neutrophilic leukocytes. Moderate fibrosis was often observed in the center of the nodular hyperplasia. This type of preneoplastic effect was not detected after the TiO₂ exposure.

Keratinizing squamous-cell metaplasias were found in the silica-exposed animals, in 5 of 50 males and 13 of 50 females. Focal or multifocal squamous cell metaplasias were also observed.

Lung tumors. An increased incidence of lung tumors was observed in the silica-exposed group. Altogether 20 primary lung tumors were found in 19 animals. Two tumors, an adenoma and an adenocarcinoma, were observed in separate areas of the lung of one male silica-exposed rat. The distribution of tumor types consisted of 3 adenomas (1 male), 11 adenocarcinomas (3 males, 8 females), 4 benign cystic keratinizing squamous-cell tumors (2 males, 2 females), 1 adenosquamous carcinoma (male), and 1 squamous-cell carcinoma (male). Thus a total of 12 lung tumors were observed among the female rats, whereas among the males 8 lung tumors were found. Details of the results are shown in table 1. With a total number of 15 out of 20 lung tumors, the prevalence of adenoid tumors was surprisingly high. With one exception, all adenoid tumors were located in the large lung lobes (lobus sinister and lobus dexter caudalis). The adenoid tumors can be separated into two different groups: one type with severe, mostly central fibrosis and the other without significant fibrosis. In the group without severe fibrosis (7 cases) a solid appearance of the tumor was found in two cases. In two other cases there was an irregular proliferation on the alveolar septae, and in three cases a central tubular structure was observed. Five of six adenocarcinomas were not clearly demarcated and showed local infiltrative growth. Three of these tumors invaded bronchi, blood vessels, or lymphatic vessels. In two adenocarcinomas, which were classified as being of a nonfibrotic tumor type and which were investigated by electron microscopy, the tumor cells showed signs of type II pneumocytes.

The adenoid tumors associated with moderate fibrosis (8 cases) showed a centrally and usually radially structured fibrosis. The epithelial component either built up small lumina, as in nodular hyperplasia, or showed tubular structures. Usually these tumors were infiltrated by polymorphonuclear neutrophil leukocytes. Two of these tumors showed an invasive growth into blood vessels and bronchi.

Discussion

The incidence of neoplastic changes found in controls and in the TiO₂-treated rats was comparable and not in excess of the historical data (7).

After the exposure of rats to a broad spectrum of tumor types was observed. These tumors were located predominantly in the

Table 1. Principal lung histopathological findings. (A = adenoma, AC = adenocarcinoma, ASC = adenosquamous carcinoma, KSCT = keratinizing cystic squamous-cell tumor, SCC = squamous-cell carcinoma)

	Number of rats investigated (equal number of males and females)	Tumor Number of rats with primary lung tumors	Number and type of tumor		Nonneoplastic lesions ^a Fibrotic foci (21–26 months)
			Benign	Malignant	
Air only	100	3	2 (A)	1 (AC)	1.2 (mild)
Titanium dioxide	100	2	1 (A)	1 (AC)	4.5 (minimal) 1.1 (mild)
Silicon dioxide	100	19 ^b	3 (A) 4 (KSCT)	11 (AC) 1 (ASC) 1 (SCC)	7.7 (mild) 92.3 (moderate)

^a Grading of the degree of fibrosis was done according to the Wagner scale: minimal, mild, moderate, severe (marked fibrosis), and severe (complete obstruction).

^b Two tumors, an adenoma and an adenocarcinoma, were observed in separate areas of the lung of one rat.

periphery of the lungs. The first tumor after silica treatment was observed after 21 months of exposure. The portion of adenoid neoplasia was relatively high compared with the total number of tumors (15 out of 20 neoplasias). The incidence of this tumor type among the females was double compared with that among the males. Similar observations have been reported by Holland et al (8).

The induction of tumors by crystalline silica has been observed in experimental animals only among rats. Intratracheally injected high doses of silica and inhalative exposure to quartz were negative among hamsters (9). A chronic inhalation study of DQ 12 quartz in hamsters and inhalation experiments with mice were also negative (10–12). Chronic persistent inflammation after quartz exposure was supposed to be an important factor in the carcinogenesis.

The results of our study can be considered significant in that the increased lung tumor incidence was observed at a chronic inhalation exposure concentration of only $1.0 \text{ mg} \cdot \text{m}^{-3}$. Tumor induction at similar low doses was recently reported by Spiethoff et al (13). These authors also used DQ 12 quartz. In most previously reported studies the exposure concentration was higher by at least a factor of 10 (9, 14).

These results confirm the classification of the International Agency for Research on Cancer, which stated that there is sufficient evidence for the carcinogenicity of crystalline silica among experimental animals (15).

References

- Muhle H, Takenaka S, Mohr U, Dasenbrock C, Mermelstein R. Lung tumor induction upon long-term low-level inhalation of crystalline silica. *Am J Ind Med* 1989;15:343–6.
- Muhle H, Bellmann B, Creutzenberg O, Dasenbrock C, Ernst H, Kilpper R, et al. Pulmonary response to tone upon chronic inhalation exposure in rats. *Fundam Appl Toxicol* 1991;17:280–99.
- Bellmann B, Muhle H, Creutzenberg O, Dasenbrock C, Kilpper R, MacKenzie J, et al. Lung clearance and retention of tone, utilizing a tracer technique during chronic inhalation exposure in rats. *Fundam Appl Toxicol* 1991;17:300–13.
- Robock K. Standard quartz DQ-12 < $12 \mu\text{m}$ for experimental pneumoconiosis research projects in the Federal Republic of Germany. *Ann Occup Hyg* 1973;16:63–6.
- Koch W, Loedding H, Oenning G, Muhle H. The generation and the measurement of dry aerosols in large-scale inhalation experiments. *J Aerosol Sci* 1986;17:499–504.
- Wagner JC. Experimental asbestosis. In: Gil J, editor. *Models of lung disease: microscopy and structural methods*. New York (NY): Marcel Dekker, 1990:761–99.
- Solleveld HA, Haseman JK, McConnell EE. Natural history of body weight gain, survival and neoplasms in the F-344 rat. *JNCI* 1984;72:929–40.
- Holland LM, Wilson JS, Tillery MI, Smith DM. Lung cancer in rats exposed to fibrogenic dusts. In: Goldsmith DF, Winn DM, Shy CM, editors. *Silica, silicosis and cancer*. New York (NY): Praeger 1986:267–79.
- Holland LM, Gonzales M, Wilson JS, Tillery MI. Pulmonary effects of shale dusts in experimental animals. In: Wagner WL, Rom WN, Merchant JA, editors. *Health issues related to metal and nonmetallic mining*. Boston (MA): Butterworth, 1983:485–96.
- Mermelstein R, Ernst H, Creutzenberg O, Dasenbrock C, Kuschner M, Mohr U, et al. Hamster response to chronic test tone inhalation [abstract]. *Toxicologist* 1992;12:839.
- Wilson T, Scheuchenzuber ML, Eskew ML, Zarkower A. Comparative pathological aspects of chronic olivine and silica inhalation in mice. *Environ Res* 1986;39:331–44.
- McNeill DA, Chrisp CE, and Fisher GL. Pulmonary adenomas in A/J mice treated with silica. *Drug Chem Toxicol* 1990;13(1):87–92.
- Spiethoff A, Wesch H, Wegener K, and Klimisch HJ. The effects of thorotrast and quartz on the induction of lung tumors in rats. *Health Physics* 1992;63:201–10.
- Dagle GE, Wehner AP, Clark ML, Buschbom RL. Chronic inhalation exposure of rats to quartz. In: Goldsmith DF, Winn DM, Shy CM, editors. *Silica, silicosis and cancer*. New York (NY): Praeger 1986:255–66.
- International Agency for Research on Cancer (IARC). *Silica and some silicates*. Lyon: IARC, 1987. IARC monographs on the evaluation of carcinogenic risks to humans, vol 42.