GENOTOXICITY

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Evidence of a no-effect level in silica-induced rat lung mutagenicity but not in fibrogenicity

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Abstract Exposure to silica can lead to fibrosis and the development of lung tumors in the rat. Based on these animal studies and on epidemiological data, silica has been classified as a human carcinogen. The initial mechanisms have not been finally clarified, but particle-induced tumor formation is at least closely associated with inflammation, the production of reactive oxygen species (ROS) and DNA damage. We investigated the dose-dependent effects of silica on the formation of the major DNA oxidation product 8-oxoguanine (8-oxo-Gua) in rat lung cells, on p53 (p53) and p53 mutant protein (p53 mut) synthesis, as well as on the amount of the surfactant phospholipids phosphatidylinositol (PI) and phosphatidylglycerol (PG) in the bronchoalveolar lavage fluids (BALF) as indicators of fibrotic processes in the lung. Rats were exposed by intratracheal instillation to various amounts of DQ12 quartz (0.15, 0.3, 0.6, 1.2, 2.4 mg/animal) and lungs were investigated after 21 and 90 days. PG decreased and PI increased quartz dose dependently. 8-oxoGua was significantly increased only after 1.2 and 2.4 mg quartz/animal. Cells expressing p53 protein were increased at 1.2 and 2.4 mg, p53 mutant protein only at 2.4 mg/animal. This indicates a no-effect level for mutagenicity at a low, but still fibrogenic quartz exposure.

Key words Quartz · Rat · 8-Oxoguanine · Mutagenicity · Fibrogenicity · No-effect level

Introduction

Based on human and animal studies, silica has been classified as a human carcinogen (IARC 1997). Human studies indicated an enhanced risk for lung cancer in cohorts, which have developed silicosis. As a consequence the risk assessment based on the relationship of silicosis and excess cancer risk is under discussion. For quantitative risk assessment it is therefore worth determining the dose response of quartz exposure for silicotic and carcinogenic effects. DNA damage is the initial event in the carcinogenic pathway (Sugimura 1992). In quartz-induced cancerogenicity, inflammation-induced reactive oxygen species (ROS) are the basic mechanisms for adverse effects at least in the rat model (Yamano et al. 1995; Borm and Driscoll 1996; Nehls et al. 1997; Bruch et al. 2000). ROS generate different types of DNA alterations including 8-oxoguanine (8-oxoGua) (Dizdaroglu 1994; Purmal et al. 1994). ROS derive from different exogenous sources but also from metabolism. Due to this continuous burden most cells possess potent defense mechanisms protecting DNA from oxidative damage. These mechanisms comprise antioxidant systems for the deactivation of ROS molecules and efficient repair proteins for the elimination of oxidative DNA damage (Jaruga and Dizdaroglu 1996; Anderson 1996). Formation of ROS and the cellular defense leads to a basic steady-state level of 8-oxoGua and other DNA oxidation products. However, high amounts of ROS can overload this cellular defense, generating much more persistent DNA alterations, which can increase the mutation rates in the genome of proliferation competent cells. Especially critical mutations are, e.g., mutations in proliferation control genes such as the p53 tumor suppressor gene (Zambetti and Levine 1993). The decrease in the PG/PI ratio is widely accepted as a marker for pulmonary fibrotic reactions, such as bleomycin-induced fibrosis (Thrall et al. 1987) and acute silicosis in the rat (Kawada et al. 1989), or cystic and idiopathic pulmonary fibrosis in humans (Gunther et al. 1989).
In this study we therefore determined the PG/PI ratio and microscopic visible fibrogenic changes in the lung tissue in correlation to low, medium and high quartz doses. The formation and persistence of 8-oxoGua and the level of p53 and a defined p53 mutation in the rat lung cells were measured to determine the dose response of quartz to DNA-damaging and carcinogenic effects.

**Materials and methods**

**Exposure**

Female Wistar rats (200 g body weight, ten animals/exposure and time point) were exposed by intratracheal instillation to 0.15, 0.3, 0.6, 1.2, 2.4 mg DQ12 quartz, to physiological saline or left untreated. At days 21 and 90 after treatment bronchoalveolar lavage (BAL) was performed on five animals, and lung tissue sections from five animals were prepared for analysis of 8-oxoGua, proliferation and p53-positive cells. The experiments complied with current German law.

Quantitative immunohistological determination of 8-oxoGua

Staining was performed as essentially described (Seiler et al. 1997), using an 8-oxoguanine affinity-purified antiserum (Nehls et al. 1997). Nuclear DNA was stained with 4,6-diamidino-2-phenylindole. Fluorescence images were quantitated by a CCD video camera and a multiparameter image analysis system as described by Seiler et al. (1997). Each value represents the average fluorescence intensity of about 100 individual cell nuclei.

p53 proteins

A sheep anti-p53–wild-type and mutant protein (pan) antiserum (Roche, Mannheim, Germany) and a mutant specific (Epitope aa 212–217) monoclonal antibody (Neomarkers, Union City, USA) were used to determine p53 protein. To quantify the immuno-

staining, at least 300 cells per section in four to six microscope visual fields were scored.

**Bronchoalveolar lavage fluids (BALF) analysis**

Analyses of the surfactant phospholipids phosphatidylinositol and phosphatidylglycerol in the BAL fluids were performed as described by Bruch et al. (1994).

**Results and discussion**

The PG/PI ratio in the BALF was decreased dose dependently after application of all quartz doses (Fig. 1).

![Graph](image1.png)

**Fig. 1** Dose- and time-dependent decrease in the ratio of PG/PI in the BALF after exposure to quartz. Values are means ± SD. Significant differences vs physiological saline were calculated using a Mann–Whitney U-test on data. Results were significantly different from controls at *P < 0.01 (**P < 0.01, ***P < 0.001)

![Graph](image2.png)

**Fig. 2** Dose-dependent increase in 8-oxoGua contents in the DNA of alveolar rat cells 90 days after instillation of quartz. Values are expressed as relative fluorescence units (rel. FU). Each value represents the average fluorescence intensity of 100 individual cells. Values are means ± SD. Significant differences vs physiological saline were calculated using a Mann–Whitney U-test on data. A value of *P < 0.05 was considered significant (*)P < 0.05, **P < 0.01, ***P < 0.001

![Graph](image3.png)

**Fig. 3** Time-dependent changes of 8-oxoGua content in the DNA of alveolar rat cells after instillation of different quartz doses. Values are expressed as relative fluorescence units (rel. FU). Significance for increase/decrease in 8-oxoGua content between day 21 and day 90 was calculated by linear regression. A positive correlation (P = 0.022) was found after 2.4 mg quartz, a negative correlation (P = 0.009) after 0.15 mg quartz treatment.