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Silica-induced micronuclei and chromosomal aberrations in Chinese hamster lung (V79) and human lung (Hel 299) cells [☆]

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Abstract

Silica is one of the most abundant and widely used mineral groups. A large number of workers are potentially exposed to one or more forms of silica. Therefore, the potential carcinogenic hazard of silica to the exposed workers is of great concern. This study examines the genotoxic potential of silica with the micronucleus and chromosomal aberration assays using cultured Chinese hamster lung fibroblasts (V79) and human embryonic lung (Hel 299) cells. One-day-old cultures were treated with two types of silica, Min-U-Sil 5 and Min-U-Sil 10, for 24 h at concentrations of 40, 80, 160 and 320 $\mu g/cm^2$. Both Min-U-Sils at 160 and 320 $\mu g/cm^2$ induced micronucleus formation in V79 and Hel 299 cells. In V79 cells, a significant increase in the micronucleus frequency was also found with 40 and 80 $\mu g/cm^2$. However, the chromosomal aberration frequency was unaffected by either Min-U-Sil 5 or 10 treatment of V79 or Hel 299 cells. Results indicated that silica, in different particle sizes, was capable of inducing micronuclei but not chromosomal aberrations in cultured animal and human lung cells and suggested that V79 cells were relatively more sensitive to silica than Hel 299 cells.

Keywords: Silica; Micronucleus; Chromosomal aberration; V79 cells; Hel 299 cells

1. Introduction

Naturally occurring silica materials have many industrial as well as non-industrial applications. They are primarily used for road building and concrete construction and are also used in foundry castings and hydraulic fracturing. Silica exposures occur in nearly all metal and non-metal mining operations, as well as during mineral processing. Workers in mills producing silica flour, by grinding and milling quartz, may also be exposed to high levels of silica dusts. The US Occupational Safety and Health Administration (1983) estimates that over 3 million workers in the United States are being potentially exposed to crystalline

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silica. Any health hazards that silica may pose to the exposed workers is an important issue.

Occupational exposure to silica (< 0.25–10 μm) is known to be associated with silicosis and other respiratory disorders (WHO, 1986). Efforts have been made to determine whether there is an association between cancer risk and silica exposure. However, no definitive conclusion thus far can be made (IARC, 1987). Results of animal inhalation studies indicate no significant differences in pulmonary adenomas in either silica exposed or control groups of mice (Wilson et al., 1986), but some silica exposed rats (2–30%) developed lung tumors (Dagle et al., 1986; Holland et al., 1983, 1986). Intratracheal administration of silica did not induce tumors in hamsters (Holland et al., 1983; Renne et al., 1985; Niemeier et al., 1986); however, 16–45% of lung tumors were observed in rats receiving silica intratracheally (Holland et al., 1983; Groth et al., 1986; Saffiotti, 1992).

Only limited short-term genotoxicity studies have been performed on silica. The available data indicate that silica is not mutagenic to *Salmonella typhimurium* (Mortelmans and Griffin, 1981). Quartz, crystalline silica, has been shown not to induce sister-chromatid exchanges in Chinese hamster lung fibroblasts (Price-Jones et al., 1980) but to induce micronuclei in Syrian hamster embryo cells (Hesterberg et al., 1986). However, no micronucleus formation was induced by silica in mice in vivo (Vanchugova et al., 1985). Whether there is any relationship between genotoxicity and particle size of silica has not been reported.

Chromosomal damage may cause a variety of adverse health effects, which may result in occupational diseases including cancer. The demonstration of micronucleus induction as an indicator of chromosomal damage has been studied in diverse tissues and organisms. Indirect evidence suggests that micronuclei can be formed as a consequence of both chromosome breakage and spindle dysfunction (Banduhn and Obe, 1985). Chromosomal aberrations are direct indicators of genetic damage caused by chemical or physical agents. Therefore, these two genetic endpoints (micronucleus and chromosomal aberrations) serve as excellent markers to assess the genotoxic and clastogenic potential of environmental or occupationally related agents. The study reported here attempts to determine the clastogenic activity of two different size distributions of silica dusts in cultured Chinese hamster lung fibroblasts (V79 cells) and human embryonic lung (Hel 299) cells using these two cytogenetic assay systems.

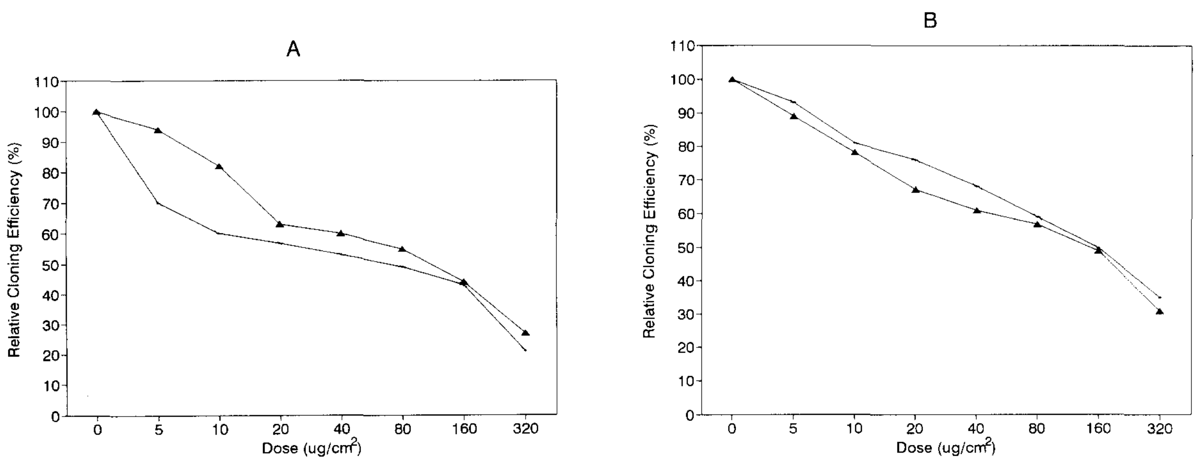

Fig. 1. Effect of different doses of silica on percent relative cloning efficiency in V79 cell cultures (A) and in Hel 299 cells (B), 5 μm silica (▲), 10 μm silica (*).

2. Materials and methods

Cell lines

V79 and Hel 299 cells used in the study were from Dr. C.C. Chang (Michigan State University, East Lansing, MI) and American Tissue Culture Collection (Rockville, Maryland), respectively. The V79 cells were grown exponentially in Eagle's minimal essential medium (MEM; Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Sigma), 1 mM glutamine (Sigma), and antibiotics penicillin (100 U/ml) and streptomycin (100 μg/ml) (Sigma). However, Hel 299 cells required special ingredients which included 0.1% lactalbumin hydrolysate in Earle's balanced salt solution (Sigma), 1 mM sodium pyruvate, and 0.1 mM non-essential amino acids (Gibco BRL, Grand Island, NY). Both cell lines were maintained in 75 cm^2 Falcon tissue culture flasks at 37° C in a humidified atmosphere containing 5% CO_2. They were subcultured twice a week using trypsin-EDTA solution (Gibco) in phosphate buffered saline (PBS). The cell cultures were periodically (every 6 months) checked for mycoplasma contamination.

Chemicals

Silica, Min-U-Sil 5 and Min-U-Sil 10, was obtained from U.S. Silica Company, Berkeley Springs, WV. Min-U-Sil 5 had a particle size distribution ranging from 0.6 to 8.0 μm with an average size of 5 μm and sizes of Min-U-Sil 10 ranged from 1 to 10 μm, mostly 10 μm range. Silica was suspended in MEM and was sonicated for 4 h for even distribution of the sample. Based on our preliminary cytotoxicity experiments (Fig. 1A,B), concentrations of 40, 80, 160 and 320 $\mu g/cm^2$ for both types of silica were used in this study.

The cytokinesis-blocking agent, cytochalasin B (CyB, Sigma) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 2 mg/ml, stored at −20° C and diluted with PBS immediately prior to use. A concentration of 4 μg CyB/ml culture medium used in the experiment was based on earlier reports (Fenech and Morley, 1985). Colcemid (Gibco) at the final concentration of 0.025 μg/ml culture medium was prepared in Hanks' balanced salt solution (HBSS) with phenol red at 10 μg/ml to arrest the cells in metaphase.

Micronucleus assay

Approximately 5×10^5 exponentially growing cells were seeded into 25 cm^2 flasks with 5 ml culture medium. One-day-old cultures were treated with silica for 24 h. The cultures were washed thoroughly with PBS and re-fed with 5 ml of fresh medium. CyB (4 μg/ml) was added to each culture dish and the cells were allowed to grow for 18–20 h. In a separate experiment, to identify the cell cycle kinetics (CCK), for Hel 299, CyB was added to the one-day-old cultures and these cultures were harvested at various time periods (8–48 h) to identify optimal harvest time to obtain binucleate cells for micronucleus analysis.

After treatment, cells were harvested using the trypsin-EDTA solution and centrifuged at $100 \times g$ for 10 min. The supernatant was discarded and the pellet resuspended with the residual solution. The cell suspensions (60–70 μl) were dropped onto each prelabeled slide and spun for 7 min at 600 rpm using a cytocentrifuge (Shandon, Pittsburgh, PA). This permitted proper spreading of the cells, maintained intact cell boundaries, and concentrated the cells in a small area on the slide. The slides were air-dried, dipped in absolute methanol for 5 s to fix the cells, and stained with Diff-Quik stain (American Scientific Products, Broadview Heights, OH). All preparations were blind coded and scored at 1000× magnification. For the CCK experiment, 200 cells were scored for the presence of 1n, 2n or > 2n, whereas 1000 binucleated cells were analyzed for the presence of micronuclei. The criteria for scoring micronuclei were similar to those of Countryman and Heddle (1976).

Chromosomal aberration analysis

After termination of silica treatment by washing thrice with PBS, cells were allowed to grow for 18–20 h. Two hours before the end of the harvest (cells trypsinized), colcemid was added to the culture. Cells were harvested, washed, and resuspended in hypotonic solution (0.075 M KCl) for 20 min at 37° C and fixed with methanol-acetic acid (3:1) three times. Three drops of cell suspension were dropped onto a cold wet slide and air-dried. The cells were stained with 10% Giemsa and 100 metaphases were analyzed for the presence of aberrations. Mitotic index was calculated based on 200 cells scored. The types of chromosomal aberrations were classified according to the standard cytogenetic procedures (Evans and O'Riordan, 1975).

Statistical analysis

All the experimental data were analyzed by the χ^2 test. They were also compared with their respective controls using a sequential linear dose-trend test for the micronucleus assay. Correlation coefficients (r) were calculated between the concentrations and micronucleated or aberrant cell frequency.

3. Results

Results from the CCK analysis of Hel 299 cells in the presence of CyB indicate that with increase in culture time, there was a decrease in the proportion of mononucleated cells (Fig. 2). The number of binucleated cells reached a peak 20 h after the addition of CyB. With further increase in time, the binucleated cells decreased and there were only a few by 48 h. Also, at 24 h and subsequent time points, cells with three or more than three nuclei were prevalent.

The frequency of micronuclei in V79 cells following silica treatment is presented in Table 1.

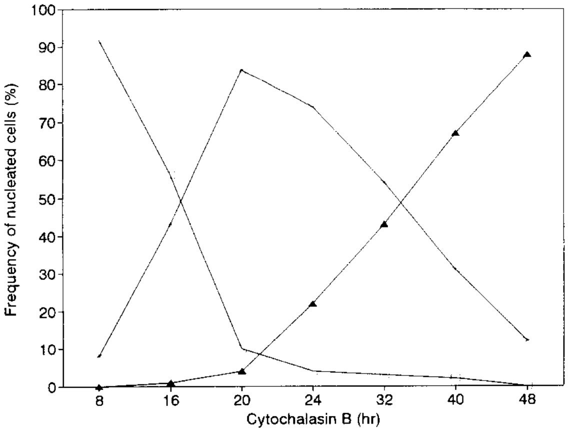

Fig. 2. Cell cycle kinetic analysis of Hel 299 cells after cytochalasin B treatment. Mononucleates (□), binucleates (∗), multinucleates (▲).

Table 1
Frequencies of micronucleated cells in V79 cultures treated with Min-U-Sil 5 and Min-U-Sil 10

Dose ($\mu g/cm^2$)	Cell cycle kinetics (%)			MNBN/1000 cells (mean ± SD)
	1n	2n	> 2n	
Min-U-Sil 5				
Control	11.5	84.2	4.3	1.5 ± 0.71
40	18.0	80.5	1.5	5.5 ± 0.70 *
80	24.8	73.3	2.0	10.0 ± 2.83 **
160	26.3	71.5	2.3	10.7 ± 1.89 **
320	18.0	80.8	1.3	22.0 ± 2.83 **
Min-U-Sil 10				
Control	11.5	84.3	4.3	1.5 ± 0.71
40	9.8	88.5	1.8	3.5 ± 2.12
80	15.0	83.0	2.0	6.0 ± 0.00 *
160	13.5	83.8	2.8	11.5 ± 2.12 **
320	16.5	81.3	2.3	15.5 ± 3.53 **

MNBN, micronucleated binucleated cells.
* $P < 0.05$; ** $P < 0.01$ compared with control using the χ^2 test.

Table 2
Frequencies of micronucleated cells in Hel 299 cultures treated with Min-U-Sil 5 and Min-U-Sil 10

Dose ($\mu g/cm^2$)	Cell cycle kinetics (%)			MNBN/1000 cells (mean ± SD)
	1n	2n	> 2n	
Min-U-Sil 5				
Control	19.5	75.8	4.8	2.0 ± 1.41
40	21.0	76.3	2.8	3.0 ± 1.41
80	28.0	68.5	3.5	5.0 ± 2.83
160	26.3	70.0	3.8	6.5 ± 0.71 *
320	28.3	68.8	3.0	8.0 ± 2.83 **
Min-U-Sil 10				
Control	19.5	75.8	4.8	2.0 ± 1.41
40	24.5	73.0	2.5	3.5 ± 0.71
80	28.0	69.0	3.0	5.0 ± 1.81
160	31.3	65.0	3.8	9.0 ± 1.85 *
320	31.5	66.3	2.3	12.5 ± 0.78 **

MNBN, micronucleated binucleated cells.
* $P < 0.05$; ** $P < 0.01$ compared with control using the χ^2 test.

Table 3
Frequency of chromosomal aberrations in Min-U-Sil 5 treated V79 cells

Dose ($\mu g/cm^2$)	Mitotic index [a]	Gaps [b]		Chromatid type [b]			Chromosome type [b]				Aberrant cells [c] (%)	Endoreduplicated cells (%)
		tg	sg	m	tb	f	cr	af	sb	d		
Control	13.0	2	0	0	0	0	0	0	0	1	3	0
40	9.5	1	1	0	1	0	0	0	0	2	5	2
80	7.5	1	1	1	0	1	0	0	0	1	5	9 *
160	5.5	1	2	0	0	0	1	0	0	2	6	14 *
320	5.0	3	3	0	1	0	0	0	0	0	7	11 *

[a] Mitotic index: number of metaphase cells/number of cells scored × 100.
[b] tg, chromatid gap; sg, chromosome gap; m, minute; tb, chromatid break; f, fragment; cr, complex rearrangement; af, acentric fragment; sb, chromosome break; d, dicentric.
[c] Based on 100 metaphase cells scored.
* $P < 0.01$, compared with control using the χ^2 test.

With increase in silica concentration, a clear dose-related increase was observed in the frequency of micronucleated V79 binucleated cells, in both Min-U-Sil 5 ($r = 0.98$) and Min-U-Sil 10 ($r = 0.97$) treated cultures. At the highest concentration (320 $\mu g/cm^2$), a 13-fold increase in total micronucleated binucleated cells over controls was noted in Min-U-Sil 5 and about a 10-fold increase in Min-U-Sil 10.

In the Hel 299 cells (Table 2), an increase in frequency of micronucleated binucleated cells was observed in both Min-U-Sil 5 and Min-U-Sil 10 treated cultures. However, the increase was significant only with the two highest concentrations tested. At the highest concentration tested, there was a 4- and 6-fold increase in micronuclei in Min-U-Sil 5 and Min-U-Sil 10 treated cultures, respectively. Min-U-Sil 10 treated cultures had greater numbers of micronuclei than those treated with Min-U-Sil 5, especially at the dose of 320 $\mu g/cm^2$. A high correlation coefficient for micronuclei ($r = 0.94$, 0.98) was observed in Min-U-Sil 5 and 10, respectively.

Results of the chromosomal aberration studies showed that the aberrations had not significantly increased by exposure to either Min-U-Sil 5 (Tables 3 and 4) or 10 (data not shown) in either V79 or Hel 299 cells over the controls. However, there were a few gaps (chromatid and chromosome) observed for both cell lines and both types of silica tested. The mitotic index decreased significantly and a dose-dependent cell cycle delay occurred in both V79 and Hel 299 cells exposed to either of the two types of silica tested. The phenomenon of endoreduplication (polyploidy) was observed only in the V79 cell line.

Table 4
Frequency of chromosomal aberrations in Min-U-Sil 5 treated Hel 299 cells

Dose ($\mu g/cm^2$)	Mitotic index [a]	Gaps [b]		Chromatid type [b]			Chromosome type [b]				Aberrant cells[c] (%)
		tg	sg	m	tb	f	cr	af	sb	d	
Control	8.0	1	0	0	0	1	0	0	1	1	4
40	5.5	0	0	1	1	0	1	0	1	3	7
80	4.5	2	2	0	1	1	0	0	0	0	6
160	4.0	3	0	1	1	0	0	1	0	2	8
320	3.5	1	3	0	1	0	1	0	0	1	7

[a] Mitotic index: number of metaphase cells/number of cells scored × 100.
[b] tg, chromatid gap; sg, chromosome gap; m, minute; tb, chromatid break; f, fragment; cr, complex rearrangement; af, acentric fragment; sb, chromosome break; d, dicentric.
[c] Based on 100 metaphase cells scored.

4. Discussion

Detection of chromosomal aberrations and micronucleus formation requires cell (nucleus) division. In cell culture, however, a certain fraction of cells do not divide. Therefore, the frequency of micronuclei in chemically treated cell cultures may be underestimated. To overcome this problem, Fenech and Morley (1985) used CyB to block cytokinesis. With this approach, micronuclei can be scored only in the dividing binucleate cells. Previous studies by others have found that the cell cycle for V79 cells is approximately 16–18 h (Bradley et al., 1981; Krishna et al., 1989; Channarayappa et al., 1990). Results of the present cell cycle kinetic study on the exponentially growing Hel 299 cells showed that the optimum time to obtain the maximum number of binucleate cells is 20 h after the addition of CyB. This time period, therefore, was used for the micronucleus assay.

The cultures treated with varying concentrations of silica (Min-U-Sils 5 and 10) displayed a dose-dependent increase in micronucleus frequencies in V79 cells. The increase in the micronucleus frequency was also observed in silica treated Hel 299 cells. These results are in agreement with previous studies which showed that silica induced micronucleus formation in Syrian hamster embryo cells (Hesterberg et al., 1986). In an in vivo study, however, silica failed to induce micronucleus formation in mice (Vanchugova et al., 1985). The disagreement between the in vitro and in vivo results may indicate that silica did not reach target cells or may be due to the inactivity of silica in the bone marrow in the in vivo situation.

In the present study, silica induced a higher frequency of micronuclei in V79 than in Hel 299 cells. A slight difference in the response was found for both cell lines to Min-U-Sil 5 and Min-U-Sil 10. However, the difference was not significant for either cell line. These results seem to indicate that V79 cells are more sensitive than Hel 299 cells to silica. It is possible that the difference is due to the difference in the capacity of phagocytosis between the two cell lines. V79 cells may be more effective than Hel 299 in the uptake of silica particles. These results also suggest that Min-U-Sil 10 particles can enter the cells as easily as Min-U-Sil 5 particles.

Under the conditions tested, neither Min-U-Sil 5 nor Min-U-Sil 10 induced chromosomal aberrations in V79 or Hel 299 cells, although the mitotic index decreased in the silica treated cultures. Sobti and Bhardwaj (1991) reported that the frequency of chromosomal aberrations in the peripheral lymphocytes of workers exposed to stone dust containing 50–60% silica was higher than that of a control population. It is not known, however, whether silica is solely, or in part, responsible for the increase in the chromosomal aberrations. The different results between chromosomal aberration and micronucleus assays seen in our study may indicate that micronucleus formation induced by silica in V79 and Hel 299 cells resulted from spindle damage caused by silica.

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