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EFFECTS OF SIMULATED PULMONARY SURFACTANT ON THE CYTOTOXICITY AND DNA-DAMAGING ACTIVITY OF RESPIRABLE QUARTZ AND KAOLIN

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Respirable-sized quartz and kaolin dusts were pretreated with simulated pulmonary surfactant dispersions of dipalmitoyl phosphatidylcholine (DPPC) in saline to model the conditioning of particles depositing in alveolar regions of the lung. DPPC-treated and untreated dusts were used to challenge lavaged rat pulmonary alveolar macrophages in vitro. Cytotoxicity was determined over a 5-d period using both total and viable cell counts from a fluorescence-based viability assay. DNA damage, as an indication of genotoxicity, was determined over a 7-d period by the single-cell gel electrophoresis assay. Untreated quartz and kaolin both expressed a significant and potent cytotoxicity, which increased with concentration and time. DPPC-surfactant pretreatment delayed significant expression of this cytotoxicity until 3 to 5 d after challenge. Untreated quartz also caused DNA damage, which increased with concentration and time. DPPC-surfactant treatment of quartz delayed most DNA damage expression to 5 and 7 d. Untreated kaolin expressed weaker activity for DNA damage, significant at the highest concentration through 5 d, and at the higher concentrations on d 7. Surfactant treatment delayed most kaolin activity for DNA damage to 7 d after challenge.

After being inhaled and upon deposition in a pulmonary alveolus, respirable particles contact the surfactant-rich hypophase on the lung surface and adsorb pulmonary surfactant before interacting with epithelial cells. Pulmonary surfactant is a complex mixture of proteins and lipids, which coats the pulmonary alveolar hypophase and respiratory bronchioles. It lowers the surface tension at the air–liquid surface interface, stabilizing the acinar lung structure from collapse. Phospholipids are a major component of pulmonary surfactant. They can reproduce in vitro many of the surface tension-modifying effects of lavaged pulmonary surfactant, but some proteinaceous components of pulmonary surfactant may be needed to facilitate the spreading of phospholipid surfactants in vivo. Pulmonary surfactant may also modify otherwise acutely toxic interactions of respired particles. Antioxidant and other components of pulmonary surfactant may be important factors in the expression or suppression of some toxic activities, but studies have shown that the phospholipid component can have

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a significant effect on the otherwise prompt membranolytic activity of respirable mineral dusts. Dipalmitoyl phosphatidylcholine (DPPC) is adsorbed from a lipid dispersion in physiological saline by quartz and kaolin particles and suppresses the otherwise prompt in vitro cytotoxicity of the dusts (Wallace et al., 1985). Acellular studies have shown that an extracellular phospholipase enzyme, PLA2, can digest DPPC from the dusts, with a consequent restoration of cytotoxic activity (Wallace et al., 1988, 1992). In vitro studies have found that cellular phagolysosomal digestion and cellular exudate digestion processes can remove quartz- and kaolin-adsorbed DPPC (Hill et al., 1995) and that in vitro cellular digestive processes can restore quartz toxicity (Liu et al., 1998).

Exposure to respirable-sized crystalline silica (quartz) dust occurs in mining, manufacturing, construction, and agriculture. It has been estimated (NIOSH, 1983) that over 3 million workers in the United States are potentially exposed to crystalline silica. Silicosis resulting from occupational exposure to quartz dust continues to cause work-related deaths (NIOSH, 1996).

Epidemiological studies have shown that the risk for respiratory cancer is increased in long-term respirable crystalline silica-exposed workers in the mineral industry (Bertazzi et al., 1986; Pairon et al., 1991). International Agency for Research on Cancer (IARC, 1997) review has concluded that there is sufficient evidence for the carcinogenicity of inhaled crystalline silica in humans. Studies of the genotoxicity of quartz indicate that it lacks mutagenic activity in *Salmonella typhimurium* and *Escherichia coli* microbial test systems (Mortelmans & Griffin, 1981). Sister chromatid exchange (SCE) induction was not found in mammalian cells (Casey, 1983). However, micronuclei (MN) were induced by silica in cultured Chinese hamster fibroblasts (V79 cells) and human lung (Hel 299) cells (Nagalakshmi et al., 1995). It also has been reported that morphological transformation in BALB/*c*-3T3 and Syrian hamster embryo (SHE) cells was induced by crystalline silica (Gao et al., 1993). Zhong et al. (1997) found that crystalline silica induced significant DNA damage in Chinese hamster lung fibroblasts as determined by the single-cell gel electrophoresis comet (SCG) assay. The SCG assay is a sensitive and rapid method for DNA damage detection in individual cells (Singh et al., 1988; Fairbairn et al., 1995). Under strong alkaline conditions, the technique can directly detect DNA single- and double-strand breaks, alkaline labile DNA base damage, and DNA repair events.

Kaolin was selected for comparison with quartz in these studies as a silicate mineral with low fibrogenic potential but with in vitro cytotoxicity comparable to crystalline silica (Vallyathan et al., 1988). Workers exposed to kaolin clay dusts—for instance, in pottery and brick production—have an increased prevalence of pneumoconiosis; however, clay mineral-induced disease typically is not as severe as that induced by quartz. There are no published reports that workers exposed to kaolin free of silica have

an excess risk of malignant respiratory diseases. The genotoxic and carcinogenic potential of kaolin has not been systematically studied in vitro, in vivo in experimental animals, or in exposed workers (Schulz, 1993).

Interactions of respired dusts with pulmonary surfactant and subsequent digestive removal processes may be critical in determining whether inhaled particles are cleared from the lung or remain and initiate fibrosis. The purpose of this study was to measure the effect of DPPC surfactant pretreatment of respirable-sized quartz and kaolin dusts on the time course of their in vitro expression of cytotoxicity and genotoxicity in cultured rat pulmonary macrophages.

METHODS AND MATERIALS

Mineral Dusts

Respirable quartz dust (Min-U-Sil 5; U.S. Silica Corporation, Berkley Springs, WV) was determined by x-ray diffraction to be 99.5% alpha quartz with 98% of particles smaller than 5 μm median area equivalent diameter. A sized fraction of respirable kaolin dust (Georgia Kaolin Mills, Augusta, GA) was used which was at least 95% aluminosilicate with no crystalline quartz detected by x-ray diffraction, with 99% of the fraction <5 μm diameter.

Surfactant

Dipalmitoyl phosphatidylcholine (DPPC; Calbiochem, San Diego, CA) was ultrasonically dispersed into 0.165 *M* NaCl (physiologic salt solution, PSS) as 5 mg DPPC/ml PSS, followed by centrifugation at 1500 × g for 10 min to remove nondispersed DPPC. Quartz and kaolin were mixed in this dispersion at a ratio of 0.1 g DPPC/g quartz and 0.2 g DPPC/g kaolin, and then centrifuged at 1500 × g for 10 min. The supernatant was discarded, and the dusts were resuspended in complete RPMI 1640 medium to desired concentrations. Prior studies of these stocks of quartz and kaolin had shown that the quartz adsorbs about 60 mg DPPC/g and the kaolin about 150 mg DPPC/g as multilayers. Approximately 20 mg DPPC/g quartz and 80 mg DPPC/g kaolin provides a bilayer covering that is stable to rinsing and fully suppresses hemolytic activity (Wallace et al., 1992).

Cells and Sample Treatment

Pulmonary alveolar macrophages were obtained by lung lavage from specific pathogen-free male Sprague-Dawley rats weighing approximately 250 g. Animals were anesthetized with sodium pentobarbital, exsanguinated at the renal artery, and lavaged with 80 ml of Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (HBSS; Gibco, Grand Island, NY). Cell suspensions were centrifuged for 10 min at 600 × g; the supernatant was discarded; and the cell pellet was resuspended in RPMI 1640 with 10% heat-denatured

fetal bovine serum (Sigma, St. Louis, MO) and 2% penicillin–streptomycin solution (Gibco). Cells of a size consistent with alveolar macrophages were counted using a hemacytometer, and their viability was determined by trypan blue dye exclusion. Cells were not differentially stained and may have included a small proportion of nonmacrophage cells.

Cytotoxicity Assay

Cells (2×10^5) were seeded in each well of a 24-well plate, and incubated at 37°C and 5% CO_2 overnight to permit cell adherence. The medium was removed by suction, and the cells were challenged with quartz or kaolin suspensions in a total volume of 2 ml. Concentrations of 20, 40, and 80 μg/cm^2 of quartz and 10, 20, and 40 μg/cm^2 of kaolin were used, both for DPPC-treated and for nontreated dusts. The dust concentrations used were extended to a lower concentration for kaolin and to a higher concentration for quartz to cover possible differences in activity associated with differences in dust surface area; there was an approximately three-to-one greater surface area of the kaolin dust compared to the quartz dust, as measured by BET nitrogen gas adsorption specific surface area measurements (Wallace et al., 1992). After incubation of cells with dusts for selected times of 1 to 5 d, the medium was transferred to a 15-ml centrifuge tube; the cells were rinsed with 2 ml phosphate-buffered saline (PBS); 1 ml of trypsin solution was added to the cell pellet; and 10 min was allowed for cells to release from the plate. Two milliliters of complete medium was added and the wells were rinsed repeatedly by pipetting the contents back and forth into the pipette; this medium was then added to the 15-ml tube. The tube was centrifuged for 10 min at 750 rpm, the supernatant discarded, and the cells resuspended in 1 ml PBS. Cell viability was determined using the Live–Dead (LD) assay (Molecular Probes, Eugene, OR). The LD reagents were warmed and 1 μl of 2 m*M* EthD-1 and 0.25 μl of 4 m*M* calcein AM were added to 3 ml PBS to make the staining solution. Then 0.1 ml of cell suspension and 0.1 ml of the LD reagent were added and mixed. The mixture was incubated for 30–45 min at room temperature. One hundred microliters of the mixture was examined by fluorescence microscopy using an excitation wavelength of 450–490 nm. Live cells are bright green and dead cells are red. The percent of live to dead cells was determined for each treatment and time point by counting 300 cells. Duplicate samples were done for each treatment and time point. Total cell number was determined using a hemacytometer with epifluorescent illumination plus a faint transmitted white-light illumination to visualize the grid lines for counting. Cell viability was calculated as the product of the percentage of live cells with the total cell number divided by the cell number at the start of the experiment.

Single-Cell Gel Electrophoresis Assay for DNA Damage

Cells (2.5×10^6) were seeded in each well of a 6-well plate and incubated at 37°C and 5% CO_2 overnight to permit cell adherence. The medium

was removed by suction and the cells were challenged with quartz or kaolin suspensions in a total volume of 4 ml. Concentrations of 10, 20, and 40 μg/cm^2 of quartz and kaolin DPPC-treated and nontreated dusts were used. Cells were incubated with dusts for selected times of 1 to 7 d; the medium was changed on d 2, 4, and 6 of treatment. Cell suspensions were centrifuged for 10 min at 4°C, 300 × g. Supernatants were discarded and cells were resuspended to an approximate density of 2 × 10^6/ml and kept at 4°C for the SCG assay.

The SCG assay was performed according to the procedure described by Tice et al. (1992), with minor modification. Fully frosted microscope slides were each covered with 100 μl of 0.5% normal melting agarose (Sigma) in Ca^{2+} and Mg^{2+}-free PBS. They were covered immediately with a 22 × 22 mm coverslip and placed on ice for 10 min to allow solidification. Then 100 μl of cell suspension was mixed with 900 μl of low-melting-point (LMP) agarose (Sigma) at 37°C, and 75 μl of the mixture (approximate 1.5–2.0 × 10^4 cells) was pipetted onto the first agarose layer (after gentle removal of the coverslip). The coverslip was replaced and the slide was maintained at 4°C for solidification. After removal of the coverslip, 75 μl of 0.5% LMP agarose was loaded, the coverslip replaced, and the gel allowed to solidify for 10 min. The coverslip was removed and the slides were immersed in freshly prepared cold lysing solution (2.5 *M* NaCl, 100 m*M* Na_2EDTA, 10 m*M* Tris, 1% sodium sarcosinate, pH 10; 1% Triton X-100 and 10% dimethyl sulfoxide v/v added just before use) for at least 1 h at 4°C. All the following steps were conducted under red light to prevent additional DNA damage. The slides were drained and placed in a horizontal gel electrophoresis tray filled with fresh alkaline EDTA buffer (300 m*M* NaOH and 1 m*M* EDTA in distilled water, pH > 13) to a level approximately 0.25 cm above the slides for 10 min to allow DNA unwinding and alkaline-labile damage expression. Electrophoresis was carried out for 50 min at room temperature at a voltage of 20 V (about 1 V/cm) and a current of 300 mA by lowering the level of the buffer. The slides were neutralized by rinsing 3 times with Tris buffer (0.4 *M* Tris, pH 7.5 with HCl) for 5 min each, then stained with 100 μl of 2 μg ethidium bromide/ml distilled water and covered again with a coverslip. Image analysis was made at 400× magnification using a fluorescence microscope (Zeiss, 7082 Oberkochen, Germany) equipped with an excitation filter of 515–560 nm from a 50-W Hg vapor lamp and a 590-nm barrier filter, coupled to a charge-coupled discharge (CCD) camera. The length of DNA migration was determined with an eyepiece micrometer by measuring the tail length (TL, distance between edge of head and end of tail) in micrometers. The eyepiece micrometer was calibrated before use. At a minimum, 50 cells per specimen, 25 cells on each of 2 replicate slides from a field of vision randomly chosen, were scored. All experiments were repeated. The results are expressed as the mean values and standard error (SE) of the mean for each data point. Statistical analysis was performed using Student's *t*-test. A difference at $p < .05$ was considered statistically signifi-

cant. Values differing at $p < .01$ are indicated in the data tables. Comparisons are made between dust-challenged cells and control cells (non-dust-challenged cells), and between cells challenged with nontreated dust and cells challenged with DPPC-treated dusts.

RESULTS

Table 1 presents the viability of pulmonary alveolar macrophages in vitro after challenge with untreated or DPPC-treated quartz. The nonchallenged control cells show a decline in measured viability from 88% at 1 d to 40% at 5 d. Quartz dust that was not surfactant treated had a deleterious effect on the cells: Viability was significantly lower than that of nonchallenged control cells for all concentrations and times except at the lowest concentration of quartz at 1 d after challenge. At d 1 and d 3, DPPC-treated quartz did not produce marked decrements in viability from those of the nonchallenged control cells. At d 5, all three concentrations of DPPC-treated quartz produced some toxicity, inducing decrements in cell viability greater than that of the nonchallenged control cells. DPPC-treated quartz induced significantly lower toxicity than native quartz at all concentrations and times except for the lowest concentration at the first time point. The general course, as detailed by the statistical data in Table 1, is that at d 1 and at d 3 the native quartz is causing cell death well above the levels seen in the nonchallenged control cells or in the cells challenged with surfactant-treated quartz. At d 5

TABLE 1. Viability of Rat Pulmonary Macrophages After Challenge With DPPC-Treated and Untreated Quartz

Exposure time (d)	Concentration (μg/cm^2)	Cell viability (%)	
		Quartz/RPMI	Quartz/DPPC
1	0	88.0 ± 11.0	85.0 ± 12.3
	20	61.7 ± 1.2	87.3 ± 16.1
	40	53.3 ± 3.7[a,c]	80.6 ± 5.2
	80	21.3 ± 3.9[b,c]	83.9 ± 6.2
3	0	64.3 ± 4.0	61.2 ± 7.2
	20	36.5 ± 3.7[b,c]	53.4 ± 2.7
	40	20.1 ± 3.7[b,c]	46.0 ± 3.0
	80	7.4 ± 1.4[b,c]	42.5 ± 2.0
5	0	40.4 ± 3.3	39.7 ± 3.2
	20	20.9 ± 0.9[b,c]	26.2 ± 0.6[a]
	40	10.2 ± 1.3[b,c]	24.7 ± 1.5[b]
	80	1.8 ± 0.3[b,c]	19.0 ± 1.8[b]

Note. Data are mean values ± standard error (SE) of the mean.
[a]Significant compared with control, $p < .05$.
[b]Significant compared with control, $p < .01$.
[c]Significant compared with quartz/DPPC at the same concentration, $p < .01$.

TABLE 2. Viability of Rat Pulmonary Macrophage After Challenge With DPPC-Treated and Untreated Kaolin

Exposure time (d)	Concentration ($\mu g/cm^2$)	Cell viability (%)	
		Kaolin/RPMI	Kaolin/DPPC
1	0	92.8 ± 2.6	93.9 ± 3.4
	10	82.5 ± 5.6	90.3 ± 4.3
	20	75.8 ± 4.4[a]	86.1 ± 1.8
	40	73.4 ± 4.5[b]	85.4 ± 7.1
3	0	78.5 ± 0.8	80.7 ± 1.9
	10	68.8 ± 1.6[b]	77.9 ± 4.7
	20	54.3 ± 2.7[b,c]	63.8 ± 2.8[b]
	40	25.4 ± 2.8[b]	27.9 ± 1.4[b]
5	0	55.6 ± 5.0	59.3 ± 4.2
	10	51.4 ± 4.7	49.7 ± 4.1
	20	44.7 ± 4.5[a]	31.9 ± 5.0[b]
	40	10.3 ± 0.5[b]	11.9 ± 1.1[b]

Note. Data are mean values ± standard error (SE) of the mean.
[a]Significant compared with control, $p < .05$.
[b]Significant compared with control, $p < .01$.
[c]Significant compared with kaolin/DPPC at the same concentration, $p < .05$.
[d]Significant compared with kaolin/DPPC at the same concentration, $p < .01$.

the surfactant-treated quartz toxicity begins to be observed as the cell survival statistically decreases from nonchallenged control cell levels.

Table 2 presents the viability of pulmonary alveolar macrophages in vitro after challenge with untreated or DPPC-treated kaolin. The nonchallenged control cells show a decline in measured viability similar to that of the controls in the silica tests, here from 93% at d 1 to 56% at d 5. Native kaolin dust, i.e, kaolin that was not surfactant treated, had a deleterious effect on the cells: Viability was significantly lower than that of nonchallenged control cells at all concentrations and times except at the lowest concentration of kaolin at d 1 and 5. For cells challenged with DPPC-treated kaolin, decrements in viability were not significantly different from those of the nonchallenged control cell at d 1, but the two highest concentrations of DPPC-treated kaolin provided significantly greater toxicity at d 3 and 5. Viabilities of cells challenged with DPPC-treated kaolin were comparable to viabilities of native kaolin challenged cells, with statistically significant difference only at the intermediate concentration on d 3. However, DPPC-treated kaolin did not significantly differ from the nonchallenged controls at d 1 while native kaolin did. The general pattern is that native kaolin is causing cell death above the levels seen in the nontreated negative control cells at all time points; that DPPC treatment of kaolin suppresses that toxicity at d 1; and that the toxic activity of DPPC-treated kaolin is seen at d 3 to d 5.

DNA damage was examined by the SCG assay, using measurements of the tail length. Untreated quartz caused a concentration- and time-dependent increase in tail length over a 7-d period (Table 3 and Figure 1). Statistically significant increases in tail length were induced at the two highest quartz concentrations at all time points, and the three highest concentrations were active at d 5 and 7. DPPC-treated quartz did not cause a significant increase in tail length at 1 d after challenge; it was active at the highest concentration on d 3; and the 2 highest concentrations were active at d 5 and the 3 highest at d 7, approaching the value for untreated quartz at 7 d. The highest concentration of native untreated kaolin induced statistically significant increases in tail length at each time point, as shown in Table 4. The two highest concentrations of native kaolin also produced statistically significant DNA damage above the nonchallenged controls at 7 d after challenge. DPPC-treated kaolin showed no statistically significant activity until d 5 at the highest concentration; the two highest concentrations of DPPC-kaolin were active at d 7.

TABLE 3. Measurement of DNA Migration Induced by DPPC-Treated and Untreated Quartz in Rat Pulmonary Macrophages With the SCG/Comet Assay

Exposure time (d)	Concentration (μg/cm^2)	Tail length (μm)	
		Quartz/RPMI	Quartz/DPPC
1	0	5.0 ± 0.7	5.0 ± 0.7
	10	5.7 ± 0.8	5.2 ± 0.8
	20	8.6 ± 1.1[b]	5.6 ± 0.8[c]
	40	10.4 ± 1.2[b]	5.9 ± 0.8[d]
3	0	6.4 ± 0.9	6.7 ± 0.9
	10	8.2 ± 1.0	6.9 ± 0.9
	20	11.1 ± 1.3[b]	7.7 ± 1.0[c]
	40	13.7 ± 1.5[b]	10.3 ± 1.2[a]
5	0	7.7 ± 0.9	8.0 ± 1.0
	10	13.6 ± 1.4[b]	9.4 ± 1.1[c]
	20	18.6 ± 1.7[b]	12.9 ± 1.3[b,d]
	40	28.1 ± 1.6[b]	16.6 ± 1.5[b,d]
7	0	11.5 ± 1.4	11.8 ± 1.9
	10	19.1 ± 1.6[b]	18.3 ± 1.6[b]
	20	25.8 ± 1.8[b]	22.3 ± 1.9[b]
	40	32.4 ± 2.1[b]	24.5 ± 1.9[b,c]

Note. 50 Cells were scored per specimen, duplicate specimen in each experiment, duplicate experiment, $n = 200$. Data are mean values ± standard error (SE) of the mean.

[a]Significant compared with control, $p < .05$.

[b]Significant compared with control, $p < .01$.

[c]Significant compared with quartz/RPMI at the same concentration, $p < .05$.

[d]Significant compared with quartz/RPMI at the same concentration, $p < .01$.

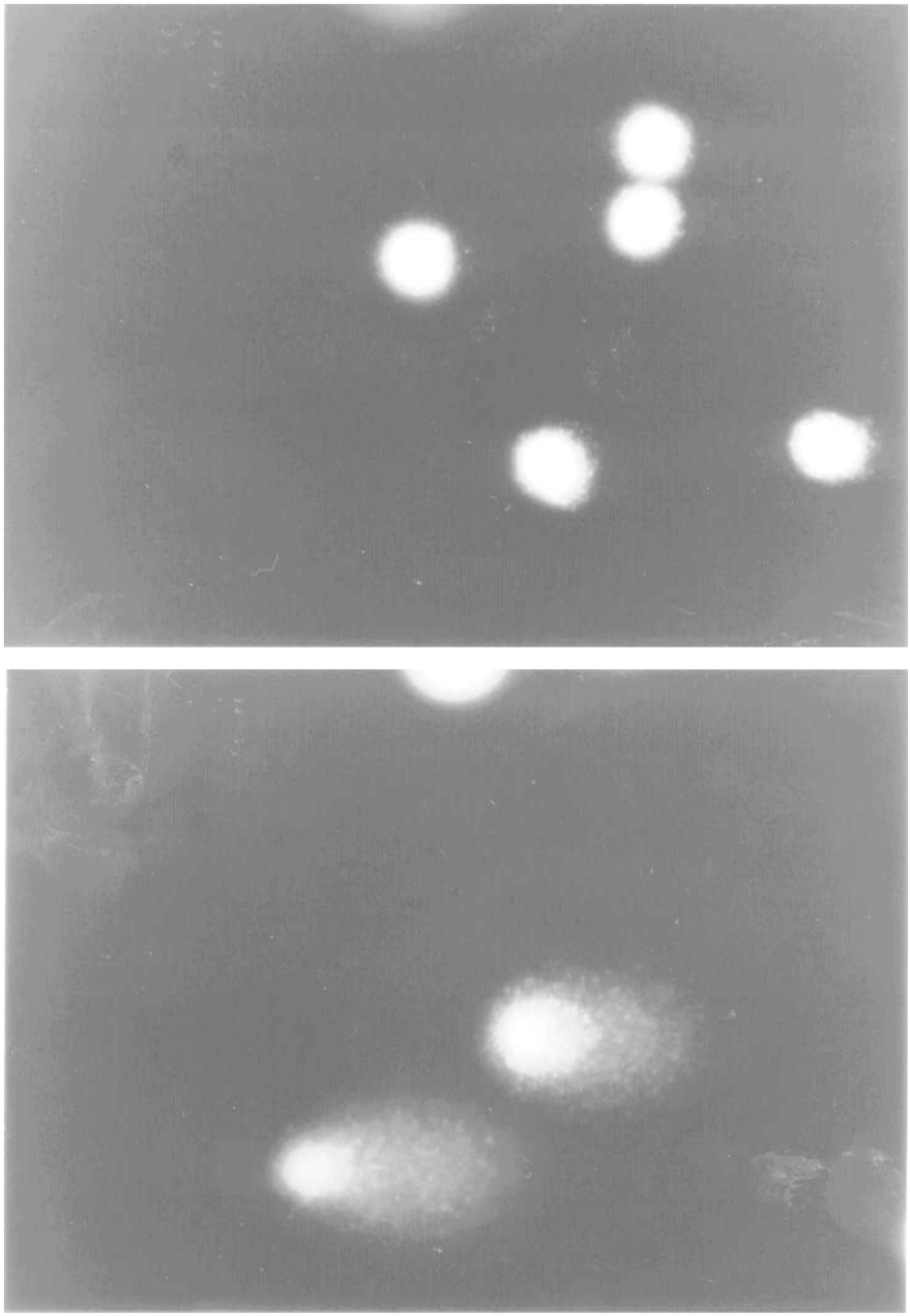

FIGURE 1. Photomicrograph (400×) of DNA migration patterns in quartz-treated rat pulmonary macrophages. (A) Untreated cells. (B) Cells exposed to quartz for 5 d at concentrations of 10 μg/cm^2.

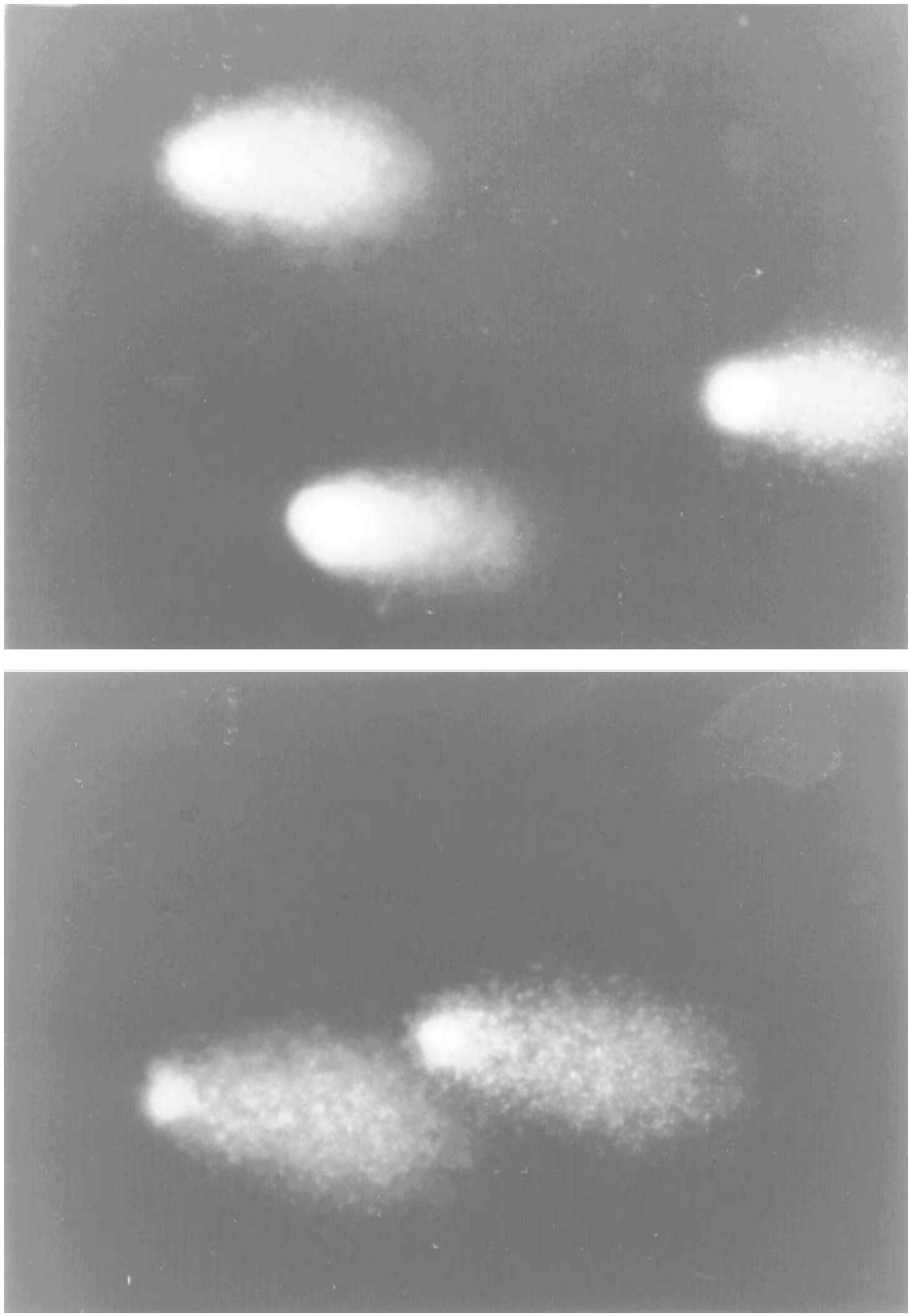

FIGURE 1. (*Continued*) Photomicrograph (400×) of DNA migration patterns in quartz-treated rat pulmonary macrophages. (C, D) Cells exposed to quartz for 5 d at concentrations of 20 and 40 μg/cm^2, respectively.

TABLE 4. Measurement of DNA Migration Induced by DPPC-Treated and Untreated Kaolin in Rat Pulmonary Macrophages With the SCG Assay

Exposure time (d)	Concentration (μg/cm^2)	Tail length (μm)	
		Kaolin/RPMI	Kaolin/DPPC
1	0	4.3 ± 0.7	4.5 ± 0.7
	10	5.0 ± 0.7	4.9 ± 0.7
	20	5.9 ± 0.8	5.3 ± 0.8
	40	8.9 ± 1.2[a]	5.7 ± 0.8[c]
3	0	5.5 ± 0.7	5.8 ± 0.8
	10	6.0 ± 0.9	6.0 ± 0.9
	20	7.1 ± 0.9	6.3 ± 0.8
	40	9.6 ± 1.2[b]	7.3 ± 0.9
5	0	8.3 ± 1.0	8.7 ± 1.1
	10	9.2 ± 1.2	8.8 ± 1.0
	20	10.7 ± 1.2	9.2 ± 1.1
	40	12.8 ± 1.3[b]	12.3 ± 1.4[a]
7	0	10.6 ± 1.2	11.1 ± 1.2
	10	13.4 ± 1.3	12.7 ± 1.3
	20	16.6 ± 1.6[b]	15.0 ± 1.5[a]
	40	22.0 ± 1.8[b]	20.2 ± 1.8[b]

Note. 50 Cells were scored per specimen, duplicate specimen in each experiment, duplicate experiment, $n = 200$. Data are mean values ± standard error (SE) of the mean.

[a]Significant compared with control, $p < .05$.

[b]Significant compared with control, $p < .01$.

[c]Significant compared with kaolin/RPMI at the same concentration, $p < .05$.

DISCUSSION AND CONCLUSIONS

Native quartz and kaolin dusts caused a concentration- and time-dependent decrement in cell viability of lavaged rat pulmonary macrophage in vitro, measured at 1, 3, and 5 d after challenge. Total viabilities were measured; that is, at each time point the viable fraction of cells in culture measured by the Live–Dead assay was multiplied by the fractional decrease in the absolute number of cells in culture. Quartz concentrations used in the viability assay ranged from 20 to 80 μg/cm^2, while kaolin concentrations used were 10 to 40 μg/cm^2. This quartz had a specific surface area as measured by nitrogen gas adsorption isotherms of 4 m^2/g; the kaolin specific surface area was 13 m^2/g (Wallace et al., 1992). The mass-concentration values used here in the cytotoxicity assay were extended for quartz over kaolin to provide a high surface area concentration of quartz comparable to (midway between) the two higher concentrations of kaolin on a surface area basis. The higher specific surface area of the kaolin indicates that a greater fraction of the kaolin mass was in smaller particle size range than for the quartz dust. It is not clearly known if dusts should be compared on a mass or particle count or surface area basis.

Comparisons of toxicities between mineral dusts should attempt to consider all three parameters, matching dusts to the extent possible and including dose ranges broad enough to see effects or make comparisons of effects that may be different functions of the different dose parameters. The effects of the dusts were compared with the background of decreasing viability with time of the nonchallenged control cells. At essentially all times and concentrations, native quartz and kaolin dusts caused losses of cell viability that were greater than those experienced by the control (nonchallenged) cells. DPPC-surfactant pretreatment of the dusts altered this expression of toxicity. The loss of viability of cells challenged with surfactant-treated dusts for 1 d was not significantly greater than that of the non-challenged control cells. This is consistent with studies of the acute suppression of in vitro cytotoxicity of quartz and kaolin by incubation of dusts with DPPC surfactant (Wallace et al., 1985, 1988, 1992). At d 5 both DPPC-treated dusts at the two highest concentrations produced decrements in viability that were significantly greater than losses in the nonchallenged control cells. This is interpreted as a partial restoration by d 5 of the dust toxicity, which initially had been suppressed by the prophylactic DPPC surfactant coating on the particle surfaces. The restoration of toxicity does not appear complete at d 5: Native quartz-induced decrements in viability still are significantly greater than those induced by DPPC-treated quartz at that time. The observed suppression of toxic activity at d 1 to 3 and the apparent beginning restoration of significant toxic activity of the DPPC-treated dusts by d 3 to 5 is consistent with other studies of the in vitro rates of surfactant digestion from quartz or kaolin particles by cellular digestion processes. In cell-free systems, phospholipase enzymes can remove adsorbed DPPC surfactant from quartz and kaolin dusts and restore dust toxicity that had been suppressed by DPPC adsorption (Wallace et al., 1988, 1992). In vitro cellular response to DPPC-treated quartz was observed over a 3-d period for pulmonary macrophage (Antonini et al., 1994). In vitro digestion of DPPC from quartz and kaolin by the macrophage-like P388D1 cell line was measured over a 9-d period (Hill et al., 1995). Parallel time courses of the in vitro removal of DPPC surfactant and restoration of quartz toxicity to pulmonary macrophage over a 7-d period have been reported (Liu et al., 1998). The current study supports the theory that phospholipid surfactant adsorption onto mineral dust surfaces suppresses their otherwise prompt cytotoxicity, and that digestive removal of this coating is associated with restoration of expression of dust toxicities. An alternative explanation for the restoration at d 3 and 5 of toxicity above nonchallenged control cells is that it is a response to nonspecific phagocytosis. Such mechanisms, e.g., a respiratory burst of oxidant production due to phagocytosis of innately nontoxic particles, would be expected to express a prompt toxicity, which is not seen here. The comparability of the no-dose control and the DPPC-treated particulate challenge in the shorter term (1 to 3 d) and their comparability at low dose of kaolin for

longer times (3 to 5 d) indicate that phagocytosis per se was not a factor in the observed toxicities in the time courses studied here. That is, challenge with DPPC-treated quartz or DPPC-treated kaolin represented, in the short term, challenge with a dust of temporarily neutralized toxicity, and this challenge displayed no effect of inert particle phagocytosis in the assays.

In the present study, DNA damage was determined by the SCG assay as an indication of genotoxicity. This technique for quantitation of low levels of DNA damage in individual cells developed by Singh and coworkers (1988) uses an alkaline-based microgel electrophoretic assay. Application to mineral dust-challenged pulmonary macrophage in this study indicated that untreated quartz caused a concentration- and time-dependent increase in DNA damage, as measured by tail length in the SCG assay, over a 7-d period. This is consistent with the report that crystalline silica caused a significant increase in DNA migration measured as tail length in both V79 and Hel 299 cells (Zhong et al., 1997). Pretreatment of quartz with DPPC delayed significant DNA damage to expression after 5 to 7 d, consistent with the effect of DPPC on quartz cytotoxicity. DNA damage by DPPC-treated quartz at d 5 was statistically greater than in the nonchallenged controls, but was statistically less than the damage induced by native quartz; that is, activity of DPPC-treated quartz was restored only partially on d 5, and was more nearly complete on d 7. Our previous studies have shown that DPPC surfactant treatment of quartz can suppress activity for micronucleus induction in V79 cells over a 7-d period (Liu et al., 1997). In the present studies, DPPC suppressed quartz genotoxic activity to pulmonary macrophage, measured as DNA strand breaks, on d 1 and 3. Restoration began on d 3 to d 5 but still was statistically lower than activity expressed by native quartz at some concentration levels at d 5 and 7.

The general pattern observed for quartz was that: (1) Significant cytotoxic and genotoxic activities were expressed promptly by native dust; (2) DPPC pretreatment suppressed those activities fully for 1 d; and (3) restoration of toxicities began significantly by d 5. For kaolin, there was prompt and significant cytotoxic behavior at d 1; and following DPPC treatment suppressed cytotoxic activity at d 1 with restoration significant at d 3. However, genotoxic activity of the native kaolin was weak at d 1, and DPPC treatment suppressed this weak activity until restoration began marginally at d 5.

In a previous study, cytotoxic and micronucleus induction response appeared to be not coupled in the V79 cell system (Liu et al., 1997), while DNA damage approximately paralleled the time course of cytotoxicity expression in this pulmonary macrophage system. This may reflect a difference between the cell types for the kinetics of phagolysosomal phospholipase enzymatic digestion of mineral-adsorbed DPPC. It also may reflect different mechanisms for genotoxic activities. Results of the pre-

sent study are consistent with, but are not exclusively interpreted by DNA damage being related to the cytotoxic process. It was noted that the concentration required for the induction of DNA migration in silica-exposed cells was much lower than that inducing a positive micronucleus response (Nagalakshmi et al., 1995).

Further studies are required to investigate these possible differences in the in vitro genotoxicity between quartz and kaolin, and to explore the time course of their expression of toxicities under conditions representative of deposition on the pulmonary alveolar hypophase. Both quartz and kaolin expressed prompt cytotoxic activity. DPPC treatment temporarily suppressed that activity. Restoration of cytotoxic activity was significant over 3 to 5 d for both dusts. In this in vitro system, the interaction of surfactant-treated dusts with lavaged pulmonary macrophages does not appear to provide a mechanistic basis for the distinct in vivo disease-inducing potentials of quartz versus kaolin dusts. That is, both dusts express toxic activities in a comparable time after macrophage cell challenge, and that time is short compared to clearance times for particles from some compartments in the lung. Distinct kinetics for expression of toxicity have been seen in cell-free phospholipase digestion studies using pH-neutral PLA2 to digest DPPC from quartz and kaolin (Wallace et al., 1992). There is evidence that critical toxic events in mineral dust-induced fibrosis occur in cells in the alveolar interstitium rather than in the macrophages of the alveolar surface hypophase (Adamson et al., 1989). That is, events that distinguish the pathogenic potentials of quartz in contrast to some other mineral dusts, such as clays, may not involve the alveolar macrophage as the definitive distinguishing target; instead, critically different effects such as differences in surfactant digestion and consequent restoration or delayed restoration of cytotoxic activity may occur for the fraction of particles in the interstitium. Measurement of surfactant removal and restoration of toxic activities under conditions of enzymatic digestion by pulmonary alveolar interstitial cells may determine if mineral-specific differences occur for cells associated with locations of fibrosis and tissue damage in vivo, such as interstitial cells with pH-neutral phospholipase activity.

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