Freshly Fractured Quartz Inhalation Leads to Enhanced Lung Injury and Inflammation

Potential Role of Free Radicals

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Silicosis is a devastating pulmonary disease that continues to occur in industrial workplaces. Its pathogenesis is under critical evaluation, and this report provides new concepts on the possible early events that occur in lungs resulting from the inhalation of freshly fractured versus aged quartz in the development of two diverse disease entities. In this study, we evaluated the biochemical and pathologic changes in the lavagate and lungs of rats exposed to freshly fractured quartz (generated by jet milling), aged quartz (milled then aged for 2 mo prior to use), or clean air 5 h a day for 10 d over a 2-wk period. The concentration of crystalline quartz in the chambers averaged 20 mg/m^3. Particle concentrations and particle size were similar for the freshly milled and aged quartz exposures. However, free radical concentrations associated with the freshly milled quartz samples were significantly higher than those for aged quartz. After a 2-wk exposure, animals were killed and studied by bronchoalveolar lavage and pulmonary histopathology. Inhalation of aged quartz increased the number of bronchoalveolar lavage cells, demonstrated histopathologic evidence of increased pulmonary infiltrates, showed enhanced concentrations of biochemical markers of lung injury, increased lipid peroxidation, and the ability of pulmonary phagocytes to produce more oxygen radicals. In general, all these pulmonary responses were significantly more pronounced after inhalation of freshly fractured quartz compared with aged quartz. In contrast, antioxidant enzymes showed decreased concentrations in the freshly fractured quartz-exposed group compared with the aged quartz–exposed animals. The combination of greater radical generation coupled with lower antioxidant concentrations may explain the augmented pulmonary inflammation and damage in response to fresh quartz dust exposure. These data suggest that those employed in occupations where inhalation of freshly fractured quartz is likely, i.e., sandblasters, rock drillers, and silica flour millers, may experience an increased oxidant burden which could lead to enhanced pulmonary injury and the development of acute silicosis. **Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR, McLaurin JL, Khan A, Teass A. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: potential role of free radicals.** AM J RESPIR CRIT CARE MED 1995;152:1003–9.

Occupational exposure to crystalline silica is associated with the development of acute silicolipoproteinosis (acute silicosis) and chronic nodular pulmonary silicosis (1–3). Acute silicosis usually occurs in occupations where silica is fractured or ground into fine powder by mechanical processes. Acute silicosis becomes clinically apparent within 2 to 5 yr after exposure and frequently results in death due to hypoxia (3). Conversely, chronic silicosis occurs in workers with prolonged exposure to dusts containing less than 18% silica with clinically apparent disease developing after 20 or more years of exposure (3).

Recent reports indicate that fracturing silica (quartz) results in the generation of silicon–oxygen radicals on the cleavage planes (4–6). In addition, we recently reported that freshly fractured quartz can react with aqueous media to generate hydroxyl radicals and that fresh quartz is biologically more reactive toward pulmonary cells than aged quartz (5, 6). Results of *in vitro* studies indicate that freshly fractured quartz is highly cytotoxic causing more cell membrane damage, greater leakage of enzymes, and more lipid peroxidation than aged quartz (5, 6). Based on these *in vitro* studies, we hypothesized that the fresh surface of fractured quartz, as generated in occupational settings such as sandblasting, rock drilling, tunneling, and silica flour mill operations, may play a significant role in the pathogenesis of enhanced acute lung injury (5).

Although *in vitro* studies consistently support this hypothesis, *in vivo* evidence is required to support its biological plausibility. Therefore, to test this hypothesis, we designed the present short-term *in vivo* study and investigated the comparative inflam-

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matory and biochemical markers of toxicity in rats exposed to freshly fractured and aged quartz of the same composition and particle size. In addition, histopathologic evaluations were carried out to assess the corresponding morphologic changes in the lung.

METHODS

Reagents

Stock crystalline αquartz of greater than 99% purity with a mass median diameter of 193 μm (geometric standard deviation 1.3) was obtained from Unimin Corporation (New Canaan, CT). The quartz was acid treated by the Unimin Corporation to remove the trace metal contamination. This acid treatment resulted in the destruction of any endotoxin present on the dust (7). Catalase, diethylene triaminepentacetic acid (DET APAC), deferoxamine mesylate, superoxide dismutase (SOD), horseradish peroxidase type IX, scopoletin, *cis*-9-*cis*-12-octadecadienoic acid (linoleic acid), and pyruvic acid were obtained from Sigma Chemical Company, St. Louis, MO. The spin trap, phenyl-*N*-*tert*-butyl nitrone (PBN), was obtained from Aldrich Chemical Company, Milwaukee, WI. Lactate dehydrogenase (LDH), catalase, and glutathione peroxidase (GPx) were measured using Roche Diagnostic reagents (Roche Diagnostic Systems, Montclair, NJ). *N*-acetyl-β-D-glucosaminidase (NAG) was estimated using diagnostic reagents (Mannheim Boehringer, Inc., Indianapolis, IN).

Inhalation Exposures

Freshly fractured and aged (60 d) quartz dust was prepared from the same stock of quartz using a Trost Gem-T Research jet mill (Model 1047; Garlock, Inc., Newton, PA) fed by a dry material feeder with a 1/4 inch diameter stainless-steel helix (Series 100; AccuRate; Whitewater, WI). The jet mill was fit with a polyurethane liner and stainless-steel jets and operated at a P-jet pressure of 70 psi and an O-jet pressure of 100 psi. For the fresh quartz exposure, the quartz aerosol passed from the mill through a BGI Aero-2 cyclone (BGI, Inc., Waltham, MA) and into a mixing chamber, where it was diluted with clean air to 20 mg/m³ before entering the exposure chamber. The aerosol was neutralized by a Pulse-Flow controller (Model PFC-20; Static Control Services, Palm Springs, CA) with an ionizing air nozzle (Model AN-6; Static Control Services) located in the clean-air stream immediately upstream of the mixing chamber.

To obtain aged quartz dust, the aerosol from the jet mill was passed through a BGI Aero-2 cyclone and into a Teflon-membrane bag (W.L. Gore Co., Elkton, MD). The quartz dust recovered from the bag was stored for approximately 60 d, except that quartz dust aged for 24 d was used on the last 2 d of exposure. This difference in the age of quartz resulted in the exposure of rats to 2.29×10^{14} spins containing quartz (a projected increase of 0.46×10^{14} spins of free radicals) over the expected radical concentration of 1.83×10^{14} spins present in the 60 d aged quartz. The aged-quartz aerosol was generated by delivering the dust from a twin-screw volumetric feeder (Model T-20; K-Tron Corp., Pitman, NJ) to the flat groove in a rotating polyurethane plate, from which it was aspirated. The resulting aerosol was delivered to the exposure chamber as aged quartz through a system identical to that used in the fresh quartz exposure.

Sixty Fischer 344 pathogen-free 8 to 10 wk old male rats were placed randomly in three groups of 20 rats for exposure to either fresh or aged quartz or to clean air. They were housed in 10 m³ (fresh and aged) or 5 m³ (control) Hinners-type inhalation exposure chambers, which were ventilated at 12 changes per hour with air. The air was cleaned by passage through a charcoal bed and a high-efficiency particle filter and conditioned to 23 ± 2° C and 48 ± 10% relative humidity. The quartz dust exposures were conducted for 10 d, 5 h each day, over a 2-wk period. Animals were killed for the study 48 h after exposure.

During the exposures, the aerosols in the fresh and aged chambers were monitored continuously by MEI RAS-2 particle sensors and were analyzed for quartz concentration by gravimetry (five pairs of consecutive samples each day). The count median circular-area equivalent diameters of aerosolized dust in the chambers were determined by scanning electron microscopy at a magnification of 1,000× with the aid of automated image analyses on eight filter samples of aged and 10 filter samples of fresh quartz dust. A total number of 8,128 particles of aged quartz and 10,161 particles of fresh quartz were analyzed from a minimum of 20 randomly selected fields of view from each sample preparation. Aerodynamic particle diameters of aerosolized dust samples were determined (four samples) using an Anderson Cascade Impactor ACFM particle sampler. Filter samples of the dust from the fresh and aged quartz exposure chambers were analyzed for free radicals, hydroxyl yield, and hydrogen peroxide. Bulk samples of the jet-milled quartz were analyzed for carbon by CO_2 yield upon oxidation and trace metals by proton-induced X-ray emission spectroscopy revealing the following average contamination by the milling process: carbon, 1100 μg/g; iron, 215 μg/g; chromium, 58 μg/g; nickel, 24 μg/g; manganese, 6 μg/g. Jet-milled bulk samples of fresh and aged quartz were analyzed for surface area measurements (four samples) using the nitrogen adsorption technique (8).

Electron Spin Resonance

Electron spin resonance (ESR) spectroscopy has long been used in investigations on the surface chemistry of quartz (4–6). In this study we used this technique to measure the intensity of ESR signal of dry quartz and for measurements of reactive oxygen species generated during phagocytosis. The quartz samples from triplicate filter samples from the exposure chambers were transferred to 5 mm quartz nuclear magnetic resonance (NMR) tubes and analyzed for free radicals (Si and Si-O) using a Varian E-109 ESR spectrometer at X-band (~9.52 GHz). All the measurements were made at a receiver gain of 1×10^4, microwave power of 50 mW, time constant of 1 s, modulation amplitude of 2 G, and a scan time of 120 s using a field amplitude of 3,380 ± 200 G. Three scans were integrated for all the samples and the scaling and analysis of the spectra were made using an EPR DAP 2.0 program. Surface radicals of the freshly fractured and aged quartz were typically centered around 2.0015 G, and surface activity was determined from peak intensities and expressed as spins/g quartz.

Hydrogen Peroxide (H_2O_2)

H_2O_2 generation by quartz exposure chamber filter samples (n = 42) was determined using a modification of the method of Lazarus and coworkers (9). The method involved the peroxidase catalyzed oxidation of 4-hydroxyphenylacetic acid (PHPA) to its dimer, a strong fluorescent compound. The modified method used in this study consisted of placing the dust-laden filter in buffer at pH 7.0, sonicating to remove the dust, and agitating and mixing to transfer the H_2O_2 from dust to solution for 30 min. The solution was centrifuged at 500 × *g* for 10 min and supernatant reacted with PHPA for 30 min at pH 10.0. Fluorescence measurements were made at an excitation of 320 nm and emission of 420 nm.

Hydroxyl Radical

Hydroxyl radical–mediated hydroxylation of 2-deoxyguanosine (2-dG) to 8-hydroxy-2-deoxyguanosine (8-OH-G) is frequently used as an indicator of DNA damage (10). Hydroxyl radical yield was measured in samples (n = 40) of quartz collected on filters from the exposure chambers using a modification of the method of Leanderson and colleagues (10) and involved no DNA or cells. The quartz dust was scraped from the filter directly in a solution of 2-dG, and agitated for 1 h to allow generation of hydroxyl radical to produce 8-OH-dG. The solution was centrifuged at 500 × *g* for 10 min and the supernatant analyzed by high-performance liquid chromatography with electrochemical detection at +0.3V.

Isolation of Alveolar Macrophages

At the termination of the 10 d inhalation and 48 h after exposure, animals were anesthetized with an overdose of sodium pentobarbital and exsanguinated. The trachea was cannulated and lungs lavaged 10 times each with 8 ml of calcium and magnesium-free Hanks' balanced salt solution. Lavagates were centrifuged at 300 × *g* and sedimented cells washed, centrifuged, and resuspended in HEPES-buffered medium containing 145 mM NaCl, 5 mM KCl, 10 mM HEPES, 5 mM glucose, and 1 mM $CaCl_2$ (pH 7.4). Total and differential cell counts were made with an electronic cell counter (Model ZBI; Coulter Electronics, Hialeah, FL) equipped with a cell sizing attachment (Channelizer C256) (11). Cell viability and purity estimates were made with the trypan blue dye exclusion technique using optical microscopy (12).

Quantitation of Enzymes

Total enzyme activity of LDH, NAG, SOD, GPx, and catalase were measured in lavage cells. Additionally, the release of these enzymes into the incubation medium was also measured as an index of cellular damage. Alveolar macrophages (AM) (1×10^6) were incubated in Hanks' buffered medium for 1 h without any added stimulus. At the end of 1 h, total enzyme activity and the amount of enzymes released into the medium were estimated using an automated Cobas Fara II Analyzer (Roche Diagnostic Systems, Montclair, NJ). The reagents and samples placed in the instrument were automatically pipetted, diluted, mixed, incubated, spectrophotometrically analyzed and calculated according to programmed instructions. LDH was measured by the increase of absorbance at 340 nm caused by the formation of NADPH according to the method of Gay and associates (13). NAG was estimated by measuring the release of 3-cresolsulfonphthalein from the substrate 3-cresolsulfonphthalein-*N*-acetyl-β-D-glucosaminide at 580 nm according to the method of Yakada and coworkers (14). SOD was estimated according to the basic method of McCord and Fridovich (15) with modifications as reported by Elstner and coworkers (16). Simultaneous measurements of inhibited and uninhibited values of cytochrome C reduction were made at 550 nm. GPx was assayed by adapting the method of Flohe and Gunzler measuring the changes in absorbance at 340 nm (17). Catalase was measured using an assay based on a method developed by Johansson and Borg (18). The purple formaldehyde adduct formed was measured by the change in absorbance at 550 nm.

Spin Trapping of Oxygen Radicals Generated during Phagocytosis

AM (5×10^5 cells/ml) obtained from animals exposed to filtered clean air, aged quartz, or freshly fractured quartz were incubated with 0.1 M PBN for 1 h at 37° C in an oscillating water bath. Control experiments were carried out without cells and without PBN. The reaction was terminated by the addition of 5 ml chloroform:methanol (2:1) and total lipids were extracted. The chloroform was evaporated in a boiling water bath and the lipid was reconstituted in 0.5 ml chloroform and transferred to NMR quartz tubes. ESR measurements of the chloroform extracts were made on the basis of a method by Vallyathan and colleagues (19) within 1 h using a Varian E-109 ESR spectrophotometer operating at X-band (9.7 GHz) using the following spectrometer settings: receiver gain 3.2×10^5; microwave power 50 mW; modulation amplitude 2 G; scan time 100 s; time constant 1 s; magnetic field 3,417 ± 50 G. ESR spectra were recorded and integrated for three scans as a measure of reactive oxygen species generated from phagocytes. Scaling and analysis of the spectra were made using the EPR DAP 2.0 program.

Lipid Peroxidation

Lipid peroxidation potential of lungs from animals exposed to filtered clean air, aged quartz, or freshly fractured quartz was monitored by measuring the thiobarbituric acid reactive substance generated during incubation of lung slices for 1 h in a buffered medium without any other stimulation according to the method of Hunter and coworkers (20). Thin lung tissue slices (300–450 mg) were incubated in phosphate-buffered medium at pH 7.4 for 1 h at 37° C in a shaking water bath. The reaction was terminated by the addition of 0.3 ml 5 N HCl and 0.625 ml 40% trichloroacetic acid. After mixing, 0.625 ml thiobarbituric acid was added to the reaction mixture, mixed, and heated in a water bath at 95° C for 20 min. The thiobarbituric acid-reactive substance developed a color which was measured after cooling at 540 nm and compared with malondialdehyde reaction with thiobarbituric acid. Malondialdehyde production was calculated from a standard graph made using the same reagents and known concentrations of malondialdehyde. Control experiments were carried out without lung tissues, with inactivated lung tissue, and in the presence of an antioxidant, butyl hydroxy toluene, to inhibit lipid peroxidation.

Bronchoalveolar Lavage Fluid (BALF)

To monitor the changes in BALF of animals exposed to clean air, aged quartz, or freshly fractured quartz, we pooled BALF from each rat after the separation of cells by centrifugation at $300 \times g$ for 10 min. The supernatant was concentrated to a 2 ml volume using a Minitran Ultrafiltration System (Millipore Corporation, Bedford, MA) and Centricon-10 microconcentrators (Amicon, Beverly, MA). This provided a 2 ml concentrated sample for each rat. LDH, NAG, SOD, GPx, and catalase were determined in the concentrated BALF according to the methods described earlier. Albumin and protein were estimated in the BALF using Sigma Diagnostic reagents and procedures.

Histopathology

For histopathologic studies, three animals from each exposure group were sacrificed by an overdose of sodium pentobarbital and exsanguination. Lungs were perfused without lavaging and fixed in 10% neutral formalin at a constant pressure of 25 cm of water for 20 min and for an additional 20 h after tying the trachea. The fixed lungs were processed for light microscopy and 4 μm sections were stained with hematoxylin and eosin. All the sections were grouped and examined blinded to the exposure protocol by a veterinary pathologist (AFH). Sections from right and left lungs were evaluated for severity and distribution of inflammation.

Statistical Analysis

For the lavagate data, analysis of variance was used to test the mean differences between control, aged, and fresh quartz-exposed groups. Student's *t* test was used to test the differences between exposed and control groups. A probability value of 0.05 or smaller was considered significant. Statistical analyses of histopathology findings were made using a commercial statistical software (Sigmastat; Jandel Scientific, San Rafael, CA). All the data are presented as means and standard deviations.

RESULTS

The time-weighted average concentrations of quartz in the exposure chambers were 22.4 ± 1.6 mg/m³ for fresh and 19.3 ± 0.3 mg/m³ for the aged dust, respectively. The count median circular-area equivalent diameter for the fresh dust was 0.46 ± 0.005 μm (range, 0.04 to 3.75) with a geometric standard deviation σg of 2.1 ± 0.1 and for aged dust 0.54 ± 0.005 (range, 0.03 to 4.33) μm with a geometric standard deviation σg of 2.2 ± 0.1. Particle size distributions of the freshly fractured and aged dust were similar and 95% of particles were within a size range of 0.15 to 2 μm mass median area diameter. The mass median aerodynamic diameters determined using an Anderson Cascade Impactor ACFM particle sizing sampler were 1.91 ± 0.09 μm (range, 1.8 to 2.1) for fresh quartz and 1.97 ± 0.16 (range, 1.8 to 2.1) for aged quartz, respectively. The Cascade Impactor measurements did not include particles smaller than 0.4 μm. The aerodynamic diameters calculated from the circular-area equivalent diameters and specific gravity of quartz showed a mean diameter of 3.4 ± 0.13 for aged and 3.0 ± 0.09 for fresh quartz respectively. The surface area measurements for the fresh quartz dust was 4.0 m²/g and for aged quartz 3.9 m²/g.

The potential of the quartz dust to generate reactive oxygen species was measured by three methods, the results from which are summarized in Table 1. The number of silicon- and oxygen-based free radicals on the quartz surface was estimated by ESR spectroscopy. Freshly milled quartz exhibits a strong ESR signal centered around 2.0015 G. Aging this milled quartz for 60 d in air decreases the number of surface radicals by 41% (Table 1). Time-weighted ESR measurements of freshly fractured quartz suggest that decay of the free radicals occurs over time at an estimated rate of 0.016×10^{14} spins per day. Freshly milled quartz also exhibits the ability to generate hydrogen peroxide and hydroxyl radicals. Production of these reactive oxygen species decreases significantly in the aged quartz samples. That is, hydrogen peroxide generation decreases by 58% while hydroxyl radical production is reduced by 20% after a 60-d aging period (Table 1).

Rats exposed to aged or freshly fractured quartz had substantial increases in pulmonary infiltrates compared with clean air–exposed animals (Table 2). Increases in the total number of cells, red blood cells, lymphocytes, and neutrophils were evident in both quartz exposure groups. Infiltration of neutrophils and lympho-

TABLE 1

POTENTIAL OF FRESH AND AGED QUARTZ TO GENERATE REACTIVE OXYGEN SPECIES

Reactive Species	Fresh Quartz	Aged Quartz
Si and Si-O Radicals,* 10^{14} spins/sample	4.40 + 0.20†	2.60 + 0.10
Hydrogen peroxide, nmol/mg	0.26 + 0.01†	0.11 + 0.01
Hydroxyl, pmol/mg	2.00 + 0.10†	1.60 + 0.10

* Data extrapolated to Day 0, the time of fracturing.
† Significantly greater than aged quartz.

cytes is indicative of an inflammatory response, whereas elevated red blood cell yields are the result of quartz-induced damage at the alveolar blood–air barrier level. It should be noted that lavage cell yields responses were significantly greater ($p < 0.05$) in rats exposed to freshly fractured quartz compared with aged quartz–exposed animals (Table 2). Indeed, freshly fractured quartz increases total cells by 119%, neutrophils by 96%, lymphocytes by 112%, and red blood cells by 253% above the levels seen for aged quartz.

Histopathologic examination of the lungs from the three exposure groups confirmed the pattern of inflammatory cellular infiltrates observed in the BAL cell analyses. Animals exposed to aged quartz show zero-to-minimal-to-mild inflammatory cellular influx with a distribution ranging from focal to minimally diffuse pattern. Exposure to fresh quartz increases the severity to minimal-to-mild with a distribution of multifocal and moderately diffuse pattern. These changes were significantly increased in rats exposed to freshly fractured as compared with aged quartz (Table 3).

Lipid peroxidation potential in both groups of quartz-exposed animals showed an increase, i.e., 125% in the aged quartz–exposed group and 209% in the fresh quartz–exposed group, compared with control animals exposed to clean air (Figure 1). Fresh quartz exhibits a significantly greater effect than aged quartz, i.e., causing 37% more lipid peroxidation than aged quartz.

Exposure to aged or freshly fractured quartz increases the albumin, protein, and NAG in the BALF. Exposure to aged quartz increases lavage albumin levels by 13% whereas freshly fractured quartz results in a 100% increase in albumin relative to the control value. For BALF NAG, aged quartz results in a 49% increase whereas fresh quartz causes a 260% increase compared with control values (Figure 2). In both cases, the response to inhalation of freshly fractured quartz is significantly higher than for aged quartz exposure. Protein in BALF of freshly fractured quartz–exposed animals showed an approximate 34% increase, whereas aged quartz–exposed animals showed only a 29% elevation above the control value.

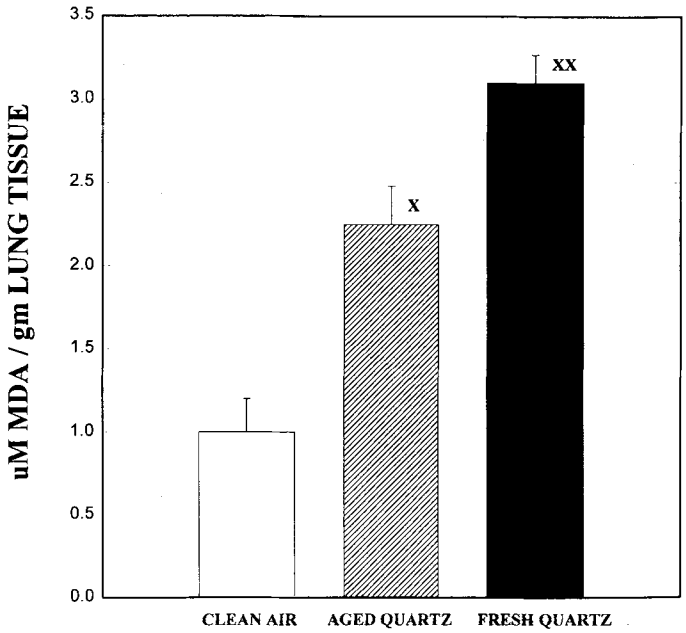

Figure 1. Lipid peroxidation potential of lung tissue slices from rats exposed to filtered clean air, aged quartz, or freshly fractured quartz. Asterisk denotes the value is significant above the filtered clean air exposure group at $p < 0.05$. Double asterisk denotes the value is significant above the filtered air group and aged quartz exposure group at $p < 0.05$.

Results of lavage cell analysis after inhalation exposure to fresh and aged quartz showed significant biochemical changes resulting from membrane injury, disruption of the integrity of lysosomes, and increased production of reactive oxygen species from bronchoalveolar lavage (BAL) cells. The release of cytoplasmic and lysosomal enzymes caused by the exposure to aged or freshly fractured quartz was substantially elevated as compared with control values. However, there were no significant differences seen between the aged quartz–exposed group and fresh quartz–exposed

TABLE 2

CHANGE IN DIFFERENTIAL CELL COUNTS OF BRONCHOALVEOLAR LAVAGES*

	Differential Cell Counts and (%) Change from Control in BAL		
Cell Type	Room Air Controls	Aged Quartz	Fresh Quartz
Total cells	$7.1 \pm 0.78 \times 10^6$	$9.3 \pm 1.2 \times 10^6$ (131)	$20.4 \pm 2.2 \times 10^6$ (286)
Macrophages	$6.7 \pm 0.69 \times 10^6$	$4.7 \pm 0.79 \times 10^6$ (70)	$5.4 \pm 0.78 \times 10^6$ (81)
Neutrophils	$\geq 0.038 \times 10^6$	$5.3 \pm 0.66 \times 10^6$ (13,847)†	$10.4 \pm 1.44 \times 10^6$ (27,479)‡
Lymphocytes	$\geq 0.038 \times 10^6$	$1.7 \pm 0.25 \times 10^6$ (4,526)†	$3.6 \pm 0.27 \times 10^6$ (9,389)‡
Red blood cells	$\geq 0.038 \times 10^6$	$1.7 \pm 0.26 \times 10^6$ (4,574)†	$6.0 \pm 0.57 \times 10^6$ (15,784)‡

* Values are means and standard errors of cell counts from five rats in each group. Figures in parentheses indicate the percent change from control values.
† Significantly greater than control values ($p < 0.05$).
‡ Significantly greater than aged quartz ($p < 0.05$).

TABLE 3

GRADING OF SEVERITY AND DISTRIBUTION OF INFLAMMATORY CELLULAR INFILTRATES IN THE LUNGS OF RATS EXPOSED TO ROOM AIR, AGED QUARTZ, AND FRESHLY FRACTURED QUARTZ*

	Room Air–Exposed	Aged Quartz–Exposed	Fresh Quartz–Exposed
Severity†	0.22 ± 0.19	0.56 ± 0.19	1.33 ± 0.58§
Distribution‡	0.22 ± 0.19	0.89 ± 0.51	3.11 ± 1.84‖

* Data are expressed as the mean ± standard deviation scores from three rats in each group.
† Severity scores were: none (0), minimal (1), mild (2), moderate (3), marked (4), and severe (5).
‡ Distribution scores were: none (0), focal (1), locally extensive (2), multifocal (3), multifocal and coalescent (4), and diffuse (5).
§ Significantly different from severity in room air–exposed rats and aged quartz–exposed rats ($p \leq 0.05$, Student-Newman-Keuls pairwise multiple comparison and one-way analysis of variance).
‖ Significantly different from distribution in room air–exposed rats and aged quartz–exposed rats ($p < 0.05$, Student-Newman-Keuls pairwise multiple comparison and Kruskal-Wallis one-way ANOVA on ranks).

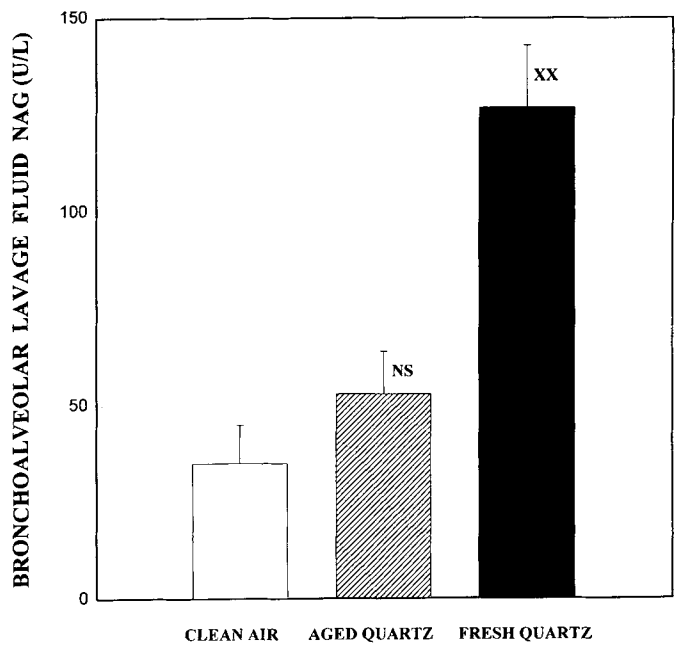

Figure 2. Bronchoalveolar fluid β-*N*-acetyl glucosaminidase levels in rats exposed to filtered clean air, aged quartz, or freshly fractured quartz. Double asterisk denotes the value is significant above the filtered air group and aged quartz exposure group at $p < 0.05$. NS = not significant.

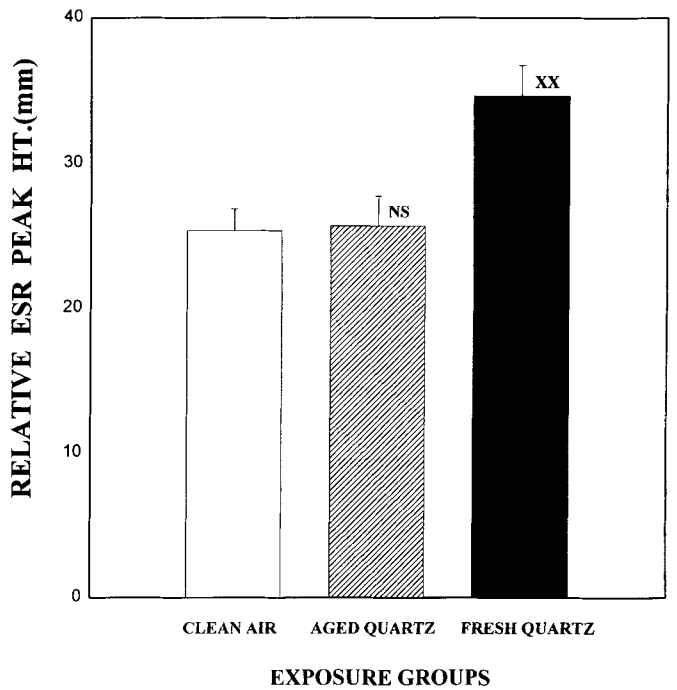

Figure 3. Relative electron spin resonance peak intensities (height peak to peak) of the reactive oxygen species signal generated in the presence of phenyl-*N*-*tert*-butyl nitrone, a nonspecific spin trap. Bronchoalveolar cells from rats exposed to freshly fractured quartz generated more reactive oxygen species. Double asterisk denotes the value is significant above the filtered air group and aged quartz exposure group at $p < 0.05$. NS = not significant.

animals. This is probably because quartz causes persistent acute biologic toxicity in the lung by repeated cycles of phagocytosis and macrophage injury. LDH, a cytoplasmic enzyme, showed approximately a 340% increase above control values in both quartz-exposed groups, and NAG, a lysosomal enzyme, showed a 255% increase in both groups. In contrast the antioxidant enzymes SOD and GPx showed significant declines in the fresh quartz-exposed rats as compared with aged quartz-exposed animals. However, all three antioxidant enzymes showed significant increases in both aged and fresh quartz-exposed animal BAL cells compared with controls. SOD showed a 67% increase, GPx showed a 176% increase, and catalase showed a 96% increase in the aged quartz-exposed group. On the other hand, SOD showed a 39% increase, GPx a 133% increase, and catalase a 77% increase in fresh quartz-exposed animals. These increases in enzyme concentrations of BAL cells in fresh quartz-exposed animals were lower than that observed for aged quartz-exposed animals, indicating a compromising of antioxidant defense in fresh quartz-exposed animals. Consistent with these findings, the BAL cells from freshly fractured quartz-exposed rats showed a 35% increase in the generation of reactive oxygen species compared with either aged quartz or controls (Figure 3).

DISCUSSION

The present study investigated several cellular, and biochemical changes, associated with functional characteristics of BALF and BAL cells after exposure to aged or freshly fractured quartz. Exposure to quartz, in general, resulted in a significant increase in the total number of inflammatory cells and erythrocytes in BAL. This increase in inflammatory cells and erythrocytes was associated with a decrease in AM. The decrease in BAL AM may be, in part, attributed to cell death, which was evident from the significant decrease in AM viability (control 89%, aged quartz 78%, fresh quartz 62%). Silica (quartz) is known to induce endothelial cell damage and cause an increase in the number of BAL neutrophils and erythrocytes (21–23). The increased lavage concentrations of red blood cells, albumin, and protein are consistent with the alveolar-capillary barrier injury as a result of exposure to quartz. The potential etiologic agent for this enhanced injury is often suggested to be reactive oxygen species (24, 25). Support for this hypothesis is evident from the results of freshly fractured quartz-exposed animal BAL cell constituents. BAL from freshly fractured quartz-exposed animals showed a 253% increase in erythrocytes and 76% increase in albumin compared with aged quartz-exposed animals. The enhanced endothelial permeability may be attributed to the increased Si and Si-O radicals in the freshly fractured quartz and the increase in neutrophils (96% increase in relation to aged quartz-exposed group) with the potential for generating reactive oxygen species. Indeed, lung lipid peroxidation is 37% higher in fresh quartz-exposed rat lungs than those from the aged quartz group. Similar increases in BAL neutrophils and erythrocytes are also reported when endothelial cell damage occurs as a result of exposure to other minerals, oxygen, or ozone (26–28).

Quantitative measurements of many components in the BALF may be useful in monitoring the disease process. Selective release of enzymes and high-molecular-weight proteins commonly not found in the BALF may provide clues to the injury and permeability of the pulmonary endothelium and alveolar epithelium. The BALF and BAL cellular enzymes showed several biochemical and functional changes which may serve as biomarkers of pulmonary injury resulting from exposure to quartz. Beck and coinvestigators (29) assayed the toxicity of several toxic and inert particulates in a hamster *in vivo* model and postulated that the LDH activity detected in the BALF is released primarily from macrophages and neutrophils. In the results presented here, we

found the cytoplasmic enzyme, LDH, and the lysosomal enzyme, β-NAG, were comparable elevated in both quartz-exposed groups, indicating persistence of cell injury to macrophages and neutrophils by repeated cycles of phagocytosis and cell death. Although there were no significant differences in the total LDH and β-NAG in BAL cells, the β-NAG showed a 211% increase in the BALF of freshly fractured quartz-exposed versus the aged quartz-exposed animals. This significant increase in the specific lysosomal enzyme β-NAG in BALF of animals exposed to freshly fractured quartz implies enhanced cell death resulting from exposure to quartz containing free radicals.

In both the aged and fresh quartz-exposed animals, a significant increase in the concentrations of antioxidant enzymes SOD, GPx, and catalase above control values was observed. These selective biochemical responses may be characterized as an outcome of oxidative stress induced by the quartz exposure. Paradoxically, all the three antioxidant enzymes are decreased in the fresh quartz-exposed group compared with the aged quartz-exposed group. This may reflect an increased consumption and/or an inability to maintain high antioxidant concentrations due to the increased demand of oxidative stress caused by the enhanced generation of reactive oxygen species in freshly fractured quartz-exposed animals. This is supported by the higher lipid peroxidation and higher generation of oxygen radicals by alveolar macrophages after fresh quartz exposure compared with aged quartz inhalation.

Acute silicolipoproteinosis is a devastating disease with an exceptionally high rate of mortality (3). In workers, acute silicosis is characterized by increases in BALF phospholipid, increases in BAL inflammatory cells (neutrophils and lymphocytes), damage at the blood-alveolar air barrier level (increased BAL red blood cells), and activation of oxidant production by pulmonary phagocytes (30, 31). Data from the present study indicate that the pulmonary reactions of rats to short duration exposure to freshly fractured quartz mimic those of acute silicosis in the following respects: inflammation, damage to the blood-air barrier, activation and generation of reactive oxygen species by pulmonary phagocytes, and increased lipid peroxidation.

Acute silicosis is commonly associated with sandblasting, tunneling, drilling, and silica flour mill operations in which crystalline quartz particles are cleaved or sheared to produce increased concentrations of surface radicals with an enhanced biological reactivity (5, 6). The results of this study are consistent with the hypothesis that enhanced generation of oxygen radicals is associated with the inhalation of freshly fractured quartz and that these radicals may contribute to the accelerated development of lung injury compared with the inhalation of aged quartz. The enhanced generation of oxygen radicals could come from four sources: (*1*) the freshly fractured quartz containing Si and Si-O radicals, (*2*) the stimulation of phagocytes resulting in a "respiratory burst," (*3*) the repeated phagocytosis resulting from cell death, and (*4*) the increased number of neutrophils. The generation of reactive oxygen species through these sources may overwhelm the antioxidant defense system to cause endothelial cell injury and edema in the lung. Data presented in this study support this hypothesis because the antioxidant enzymes, SOD and GPx, in the freshly fractured quartz-exposed group showed a significant decrease compared with the aged quartz-exposed group; this is consistent with increased consumption of these antioxidant enzymes. In addition, the lung tissue from the freshly fractured quartz-exposed group showed an increased potential for lipid peroxidation as reflected by the generation of thiobarbituric acid reactive substances. Furthermore, enhanced generation of reactive oxygen species by alveolar phagocytes harvested from freshly fractured quartz-exposed animals determined by ESR provides mechanistic support of this hypothesis.

The unique features elicited by the greater magnitudes of pulmonary inflammation, elevated concentrations of biochemical markers of cell injury in BAL fluids and cells are confirmatory indicators of a greater level of cellular damage and death induced by freshly fractured quartz containing more oxygen free radicals. It is important to recognize that histopathologic changes observed in the lungs were not dramatic in either groups of quartz-exposed animals. However, the increased pulmonary inflammation and biochemical alterations induced in fresh quartz-exposed animals has set a stage for augmented lung damage as is evident from the 77% increase in albumin above the aged quartz-exposed animals. The leakage of albumin into the BAL is often considered an index of lung injury (32) and may be associated with the alveolar lipoproteinosis. The endothelial cell injury and resulting permeability is a precursor to the microscopically visible interstitial edema occurring in acute silicosis. The enhanced oxidative stress is well illustrated by the upregulation of antioxidant enzymes SOD, GPx, and catalase. Similar upregulation of antioxidant enzymes in oxidative stress induced by mineral dust has been reported by other investigators (33–35). Among the three antioxidant enzymes studied, SOD and GPx showed significant upregulation, whereas catalase showed an insignificant trend toward slightly elevated values. This is probably due to selective upregulation of antioxidants, because upregulation of antioxidants is controlled by many intrinsic factors including cytokines. Furthermore, upregulation of various antioxidants is often not coordinated because each is determined by specific mRNA and gene expression. The enhanced exacerbation of lung injury evidenced by the increased biochemical markers in BALF may have been induced by lipid peroxidation which in turn was promoted by increased radicals present on the freshly fractured silica.

In conclusion, we believe that the enhanced generation of highly reactive oxygen species from the combined sources of phagocytes and freshly fractured quartz is likely to be one mechanism enhancing the inflammatory response and enhanced lipid peroxidation of cell membranes which may progress to acute silicolipoproteinosis. These observations are consistent with the BAL cell studies reported in human acute silicosis (31). The enhanced generation of reactive oxygen species may also promote a proinflammatory effect resulting in the release of cytokines, growth factors, and other mediators culminating in a complicated disease process.

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