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CHARACTERISTICS OF THE ACUTE-PHASE PULMONARY RESPONSE TO SILICA IN RATS

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Exposure to silica, a cytotoxic and fibrogenic mineral dust, has been demonstrated to cause pulmonary inflammation and damage to the lung tissue. In contrast to the long-term consequences, little information exists on the sequence of inflammatory/damaging events occurring acutely after exposure to silica. The purpose of this study was to determine the minimum time after the administration of silica that the inflammatory/damage response is detectable and the temporal relationship of these processes. Male Fischer 344 rats were dosed intratracheally with silica (2.5 or 10 mg/100 g body weight) or saline vehicle. At 2 and 4 h after instillation, both cellular (total cell count and neutrophil count) and biochemical (total protein, albumin, and β-glucuronidase and lactate dehydrogenase activities) parameters of inflammation and damage were evaluated in the bronchoalveolar lavage fluid. At 2 h, total protein levels were elevated at both silica doses, but all other parameters were unchanged; however, 4 h after silica exposure all parameters were elevated over those of the saline control. In a further attempt to characterize the inflammatory/damage processes, luminol-dependent chemiluminescence (LDCL) was performed on aliquots of chopped lung. At 2 h after silica instillation, phorbol myristate acetate-stimulated lung tissue from silica-treated rats had no increase in light production when compared to controls, whereas after 4 h there were significant increases in LDCL activity in both dose groups when compared to controls. The addition of superoxide dismutase (SOD) decreased LDCL activity of the 2.5 mg/100 g group by 59% (2 h) and 66% (4 h), and of the 10 mg/100 g group by 49% (2 h) and 73% (4 h). Alternatively, the addition of N-ω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, decreased the 2.5 mg/100 g group by 52% (2 h) and 60% (4 h). The 10 mg/100 g group was decreased by 67% (2 h), but only exhibited a 12% reduction at 4 h. SOD and L-NAME also inhibited the background LDCL in saline-treated rats. These reductions in LDCL activity indicate that reactive oxygen and nitrogen species play a role in the acute phase pulmonary response from silica. The results of this study indicate that the initial stages of damage begin to appear by 2 h, but damage and inflammation are definitive by 4 h after administration of silica in rats.

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Respirable particulate matter is an occupational concern because of the potential toxicity posed to the lungs. Damage to the lung infrastructure can lead to a decrease in ability and/or efficiency of oxygen exchange. Silica dust is generated secondarily from various types of mining and from sand-blasting. Upon inhalation and deposition of the silica into the respiratory tract, attempts by the body's natural defense mechanisms to remove the silica occur. Phagocyte-mediated clearance of these particles from the lungs occurs very slowly, if at all, thus allowing sufficient resident time for toxicities to be expressed (Henderson et al., 1992). Unlike most other particulate matter, silica is not detoxified by macrophage phagocytosis, and therefore the longer the silica is allowed to persist in the lungs, the more marked are the toxicities (Heppleston, 1982; Brown et al., 1989; Vallyathan et al., 1992).

As a known cytotoxic and fibrogenic dust, silica has been demonstrated to cause pulmonary inflammation (increases in numbers of one or more cell types, particularly macrophages, neutrophils or lymphocytes; Khan & Gupta, 1991) and damage to the lung tissue. This inflammation and damage may ultimately manifest itself as the fibrotic lung disease, pulmonary fibrosis (Heppleston, 1982; Driscoll et al., 1991; Driscoll & Maurer, 1991; Adamson et al., 1992). Depending upon the dose, the appearance of fibrosis can occur as rapidly as 2 wk after the introduction of silica (Reiser et al., 1982). This fibrosis is characterized by a rapid increase in the rate of lung collagen synthesis followed by an increase in the deposition of excess collagen. This collagen is abnormal with respect to normal lung collagen in that it has altered amino acid cross-links and has thus been termed "fibrotic collagen" (Last & Reiser, 1985). Lesions of this fibrotic collagen appear as whorled, rounded nodules of concentric layers of tissue around an acellular center (Lapp & Castranova, 1993).

In cases of silicosis the participation of cells and mediators in the ensuing development of lung disease is not completely understood. There is evidence that multiple mechanisms may be involved in silicosis, including (1) direct actions of the surface properties (Nolan et al., 1981; Vallyathan et al., 1988), (2) silica-dependent calcium translocation into cells (Chen et al., 1991; Van Dyke et al., 1993), (3) macrophage activation and release of oxidants (Menzel, 1992; Castranova et al., 1993; Slade et al., 1993; Van Dyke et al., 1994), (4) macrophage stimulation and secretion of chemotactic factors (Gadek et al., 1980; Lapp & Castranova, 1993), and (5) macrophage stimulation and release of cytokines that modulate inflammatory reactions (Driscoll et al., 1991; Donaldson et al., 1992; Stein & Keshav, 1992).

Although the more chronic (24 h postexposure to the point of fibrosis) consequences of silica exposure have been well characterized, little is known of the characteristics that occur very early after silica exposure. If the acute phase is critical to the development of the resultant toxicities, one would need to establish when the acute phase actually occurs and the characteristics of the acute response. The objective of this study was to determine the minimum time after the presentation of silica by intratracheal instillation that the

inflammatory/damage response is detectable, and the temporal relationship of the biochemical, cellular, and functional changes that can be observed.

The present study utilizes the sometimes controversial and "nonphysiological" intratracheal instillation procedure as opposed to inhalation exposure. Henderson and colleagues have addressed the relative merit of each procedure (Henderson et al., 1995). The studies encompassed intratracheal instillations as well as inhalation of silica and the less toxic dust titanium dioxide. These studies illustrated that the degree of alveolitis, as evaluated by histopathology and bronchoalveolar lavage fluid (BALF) analysis, was similar by the two methods of exposure. It was concluded that, in general, the relative potentials of the two materials to produce bronchoalveolitis and/or granulomatous lesions could be appropriately evaluated using either intratracheal or inhalation exposures.

MATERIALS AND METHODS

Materials

Crystalline Min-U-Sil silica (U.S. Silica Corp., Berkeley Springs, W. Va.) was a gift from Dr. Val Vallyathan, National Institute for Occupational Safety and Health, Morgantown, W. Va. The silica was cleaned by boiling in 1.0 *M* HCl for 60 min to remove any contaminants. Purity of the silica was determined by automated x-ray diffractometer and was 99.5% α-quartz. A size fraction <5 μm in diameter was made by a centrifugal airflow particle classifier. Ninety-eight percent of this fraction was <5 μm in size with a median area equivalent diameter of 3.5 μm as estimated by scanning electron microscopic image analysis. Enzyme reagents, superoxide dismutase (SOD), and *N*-ω-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Other chemicals used in the study were from Fischer Chemical Co. (Pittsburgh, Pa.).

Animal Treatment

Male Fischer 344 rats (Hilltop Lab Animals, Scottdale, Pa.) weighing 200–250 g were housed in wire cages in groups of six and allowed at least 1 wk for acclimation after arrival from the supplier. Rats were given a conventional laboratory diet (Purina Chow pellets) and tap water ad libitum.

Intratracheal Instillation Rats were treated in a manner similar to that outlined by Brain et al. (1976). After being anesthetized with Brevital (sodium methohexital, Eli Lilly & Co., Indianapolis, Ind.; 0.7 ml of a 10 mg/ml solution) i.p., rats were placed on their backs on a slanted board suspended by a wire under their maxillary incisors. The tongue was moved and the trachea transilluminated using a modified fiberoptics laryngoscope. A ball-tipped 20-gauge animal feeding needle fitted with an insulin syringe was placed into the trachea via the mouth. The silica particles were prepared at concentrations such that rats received 2.5 or 10 mg/100 g body weight in 0.5 ml of sterile normal saline solution (SNSS). Before instillation the silica suspension

was sonicated for 10 min. A 0.5-ml aliquot of silica was slowly instilled into the trachea. In control groups the same volume of SNSS (0.5 ml) was instilled. The animals remained in the slanted position for 1 min after the instillation to facilitate deposition and distribution in the lungs. Rats were observed until consciousness was regained. After recovery from anesthesia, the animals were returned to animal quarters until the time for analysis.

Bronchoalveolar Lavage Rats were anesthetized with 0.7 ml of sodium pentobarbital (Butler Company, Columbus, Ohio; 64.8 mg/ml) and exsanguinated by severing the abdominal aorta. The trachea was cannulated with PE160 tubing and the cells from the lungs were collected by BAL. While massaging the lungs, 2.0 ml/100 g body weight of warm Ca^{2+}/Mg^{2+}-free Hanks balanced salt solution (HBSS) was instilled into the lungs. For the first instillation HBSS remained in the lungs for 30 s, was withdrawn, and then was reinstilled for another 30 s to maximize retrieval of biological markers (Lindenschmidt et al., 1990). After the second withdrawal the HBSS was saved for analysis in a separate tube. For additional lavages, 5.0-ml aliquots of HBSS were sequentially instilled, withdrawn, and placed in centrifuge tubes until ~80 ml was obtained. All tubes from each rat were centrifuged at 800 × g for 7 min. The supernatant from the first lavage was transferred into a separate conical tube for further analysis. All other supernatants were discarded. Cell pellets were resuspended in 5.0 ml HBSS and the cells from each animal were pooled. The cells were recentrifuged, as already described, and the pellets were resuspended in 1.0 ml HBSS for cell counting and further analysis.

Cellular, Functional, Biochemical Assays

Cell Counts and Viability Cells harvested from lavage procedures were counted via a hemacytometer. Concurrently, cell viability was assessed by trypan blue exclusion.

Differential Cell Counts For cellular differentiation, cells (1.5×10^5) were spun in a Shandon cytocentrifuge at 400 rpm for 4 min and allowed to affix to a microscope slide. Slides were stained with Wright-Giemsa Sure Stain. Cell types (alveolar macrophages, neutrophils, and lymphocytes) were differentiated under a light microscope by counting at least 200 cells per slide.

Luminol-Dependent Chemiluminescence Luminol was incorporated into the reaction cuvettes as an amplifying agent of chemiluminescence to study the oxidative activity of the lung tissue. Luminol is first oxidized to an intermediate that subsequently converts to an aminophthalate product and the release of a photon (~425 nm). Luminol is felt to react primarily with superoxide, nitric oxide, and their reaction product peroxynitrite (Radi et al., 1993). Luminol-dependent chemiluminescence (LDCL) was measured with a six-chambered Berthold LB 9505C luminometer. LDCL was followed for 20 min at 37°C. The integrated response was determined by an accompanying computer equipped with a KINB program that was supplied with the luminometer.

Chemiluminescence is a functional assay to assess release of oxidants from cells or tissue.

Nonlavaged Chopped Lung LDCL Separate groups of identically treated animals were used for LDCL determinations; all lobes of the lungs were surgically removed and placed on a McIlwain tissue chopper, equipped with a micrometer dial, and chopped into cubes approximately 0.5 × 0.5 × 0.5 mm. Cubed portions of the lung (150 mg) were placed in a standard luminometer cuvette containing HEPES buffer. Luminol was used as an amplifier of the chemiluminescence. It was dissolved in dimethyl sulfoxide (DMSO), then diluted in physiological HEPES buffer (0.1 *M*, pH 7.4). The final concentration of the luminol in the cuvette reaction mixture was 10^{-5} *M*. Phorbol myristate acetate (PMA) was used as a soluble stimulant at a final cuvette concentration of 2×10^{-6} *M*. In each run an unstimulated baseline value was obtained by omitting the PMA from one cuvette. Inhibitors of LDCL were also used in designated cuvettes. Superoxide dismutase (SOD, the enzyme responsible for the breakdown of superoxide anion) or *N*-ω-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor that leads to a reduction in the formation of nitric oxide) was included in designated cuvettes. SOD at a final concentration of 0.2 mg/ml or L-NAME at a final concentration of 1 m*M* was added in a volume of 100 µl. The final volume in each reaction cuvette was 500 µl (150 mg tissue, 100 µl luminol, 100 µl PMA, 100 µl SOD or 100 µl L-NAME, and 50 µl HEPES). When PMA and/or the inhibitors were excluded from the cuvettes, the volume was brought up to 500 µl with HEPES buffer. All cuvettes were incubated in a 37°C water bath for 10 min prior to PMA stimulation and being placed in the luminometer.

BALF Total Protein Total protein was assayed to assess the release of proteins into the airspace in response to silica. The total protein content of the acellular fraction of fluid obtained by the first lavage was determined by a modified version of the Lowry method published by Hartree (1972). Absorbance was determined at 650 nm on a spectrophotometer. Quantification of protein was accomplished by comparison to a bovine serum albumin standard curve.

BALF Albumin Albumin was assayed to assess damage/permeability of the alveolar–capillary barrier and is specific for assessing this damage since it is a blood-derived protein. The albumin content of the acellular fraction obtained by the first lavage was determined by a modified version of the Sigma Diagnostics albumin reagent kit (procedure 631). Absorbance was determined at 628 nm on a spectrophotometer. Quantification of albumin was accomplished by comparison to a bovine serum albumin standard curve.

BALF β-Glucuronidase BALF β-glucuronidase (β-glu) is a lysosomal enzyme and was assayed to assess phagocytic cell activation/damage. The activity of β-glu in the acellular fraction of the first lavage was analyzed according to the method of Lockhard and Kennedy (1976). The absorbance was deter-

mined at 400 nm. The activity of the enzyme was calculated by using a known extinction coefficient and was expressed in units of nmol/min/ml.

BALF Lactate Dehydrogenase Lactate dehydrogenase (LDH) is a cytosolic enzyme and was assayed to assess general cell death. The activity of LDH in the acellular fraction of the first lavage was analyzed according to the procedure set forth by Wroblewski and LaDue (1955), and monitored at 340 nm in a dual-beam scanning spectrophotometer. The enzyme activity was calculated by using a known extinction coefficient and was expressed in units of μmol/min/ml.

Statistical Analysis

All parameters were analyzed by two-way analysis of variance. Repeated-measures analyses were used for all chemiluminescence data, whereas all other data were completely randomized. Effects found to be significant were analyzed further by Tukey's protected *t*-test, a multiple-comparison procedure used to determine significant differences between pairs of groups. The criterion for significance between groups was $p < .05$. All statistical analyses were carried out using GB-STAT software.

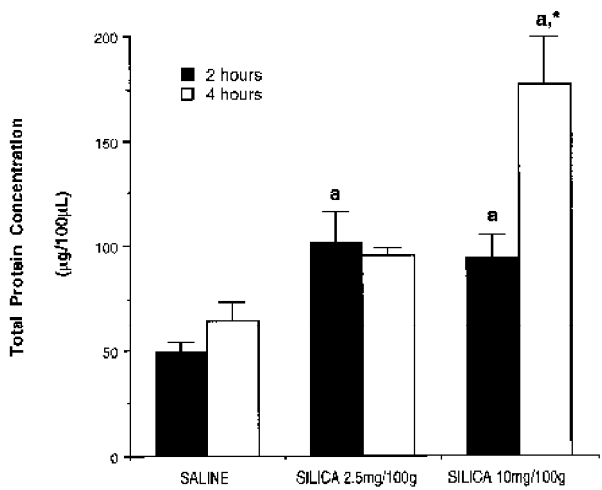

FIGURE 1. Total protein concentration in the acellular BALF from the lungs of rats 2 and 4 h after saline (control) or silica instillation. Values are means ± SEM (n = 5–6). Letter (a) denotes significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Within time or dose groups, different denotations represent significant differences from each other and the saline control.

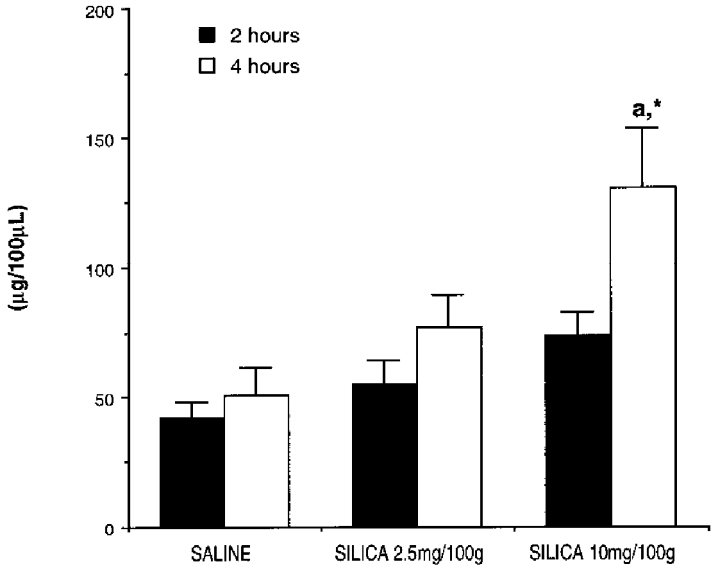

FIGURE 2. Albumin concentration in the acellular BALF from the lungs of rats 2 and 4 h after saline (control) or silica instillation. Values are means ± SEM (n = 5–6). Letter (a) denotes significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Within time or dose groups, different denotations represent significant differences from each other and the saline control.

RESULTS

Biochemical and Cellular Assessments of BALF

Treatment with silica at the two doses used in this study caused significant increases in the total protein levels in the acellular BALF 2 and 4 h after exposure (Figure 1). Regardless of the silica dose, protein levels at 2 h were elevated 2-fold when compared to saline control, whereas levels at 4 h were elevated 2.5-fold at the 10 mg/100 g dose. Additionally, at the 10 mg/100 g dose, protein levels were significantly elevated (2-fold) in a time-dependent fashion when comparing the 2-h versus the 4-h groups.

In contrast to total protein, albumin, the specific index of alveolar-capillary damage, showed no increases at either silica dose at 2 h, but was significantly increased 2.5-fold in the 10 mg/100 g dose group at 4 h (Figure 2). This elevation in albumin was also 2-fold greater than levels obtained at 2 h with the same silica dose.

The 2-h β-glucuronidase activity in the acellular BALF at both doses of silica was no different than saline control animals. At 4 h, both silica doses (2.5 and 10 mg/100 g) significantly increased levels of β-glucuronidase activ-

ity, 2- and 4-fold, respectively, within the acellular BALF when compared to the saline control group (Figure 3).

Similar to the β-glucuronidase data, lactate dehydrogenase activity within the acellular BALF at 2 h was not different from the saline control, but was significantly elevated 2- and 6-fold for the 2.5 and 10 mg/100 g dose groups, respectively, at the 4-h time point when compared to their corresponding saline control groups (Figure 4).

Mean BAL cell counts are presented in Figure 5. At 2 h after exposure to silica, macrophage and neutrophil numbers fluctuated with neither being significantly different when compared to the saline control groups (Figure 5A). At the 4-h time point after silica exposure, macrophage numbers fluctuated but again were not different from the saline control (Figure 5B). However, at this time point there was a significant increase in the total cell number at the 10 mg/100 g silica dose, due primarily to the greater than 7-fold increase in recoverable neutrophils. The increased number of neutrophils at the 4-h time was also significantly elevated 4.5-fold over that of the 2-h counterpart. Lymphocyte numbers were not affected by any treatment at either time.

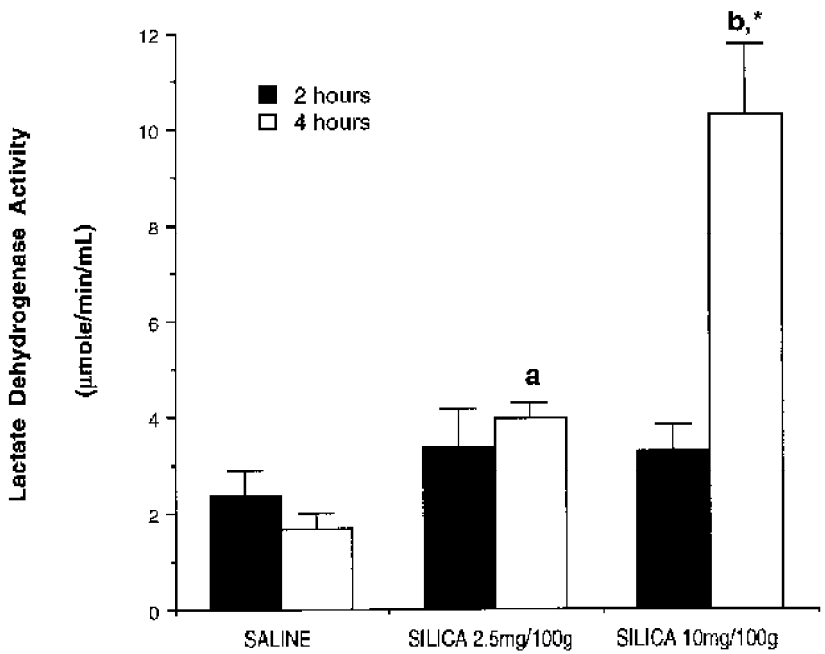

FIGURE 3. β-Glucuronidase (β-glu) activity of the acellular BALF from the lungs of rats 2 and 4 h after saline (control) or silica instillation. Values are means ± SEM (n = 4–6). Letters (a, b) denote significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Within time or dose groups, different denotations represent significant differences from each other and the saline control.

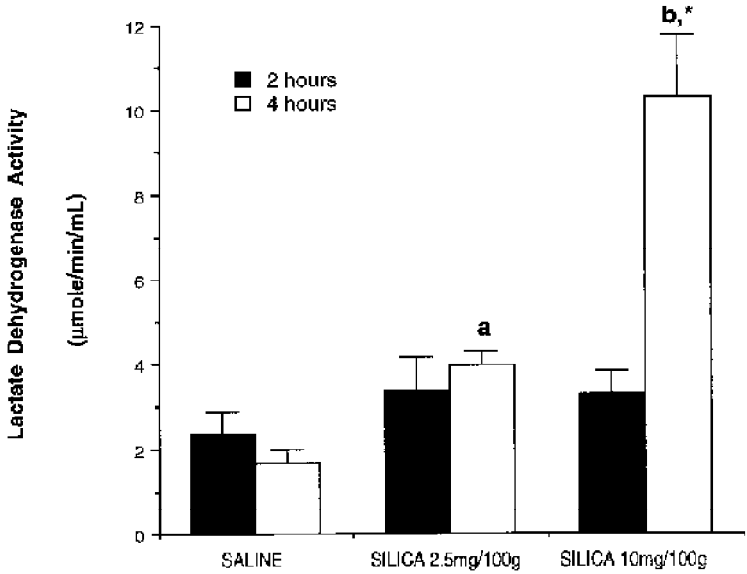

FIGURE 4. Lactate dehydrogenase (LDH) activity of the acellular BALF from the lungs of rats 2 and 4 h after saline (control) or silica instillation. Values are means ± SEM (n = 5–6). Letters (a, b) denote significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Within time or dose groups, different denotations represent significant differences from each other and the saline control.

Functional Assessment of Lung Tissue Inflammation

The integrated response of lung tissue LDCL is presented on Figure 6. When unstimulated and PMA-stimulated LDCL of lung tissue were measured, all treatments at 2 h postexposure were not different from the untreated baseline and saline control animals (Figure 6A). However, at 4 h, lungs demonstrated tremendous increases in LDCL activity (Figure 6B). Both the 2.5 and 10 mg/100 g doses of silica induced significant increases, 4- and 7-fold, respectively, in the production of light when stimulated with PMA, as compared to controls and their 2-h counterparts. Also evident was the significantly enhanced light production from unstimulated lung tissue from the 4-h 10 mg/100 g dose animals (4-fold over saline control and 6-fold over the 2-h counterpart).

Addition of SOD, which catalyzes the breakdown of superoxide anion, greatly inhibited the PMA-stimulated LDCL response of the lung tissue (Table 1). Preincubations of SOD with the lung tissue for 10 min inhibited LDCL light in the 2.5 mg/100 g group by 58.9% (2 h) and 66.2% (4 h), and in the 10 mg/100 g group by 49.3% (2 h) and 73.1% (4 h). When L-NAME, a nitric oxide synthase inhibitor, was preincubated with the lung tissue for 10 min,

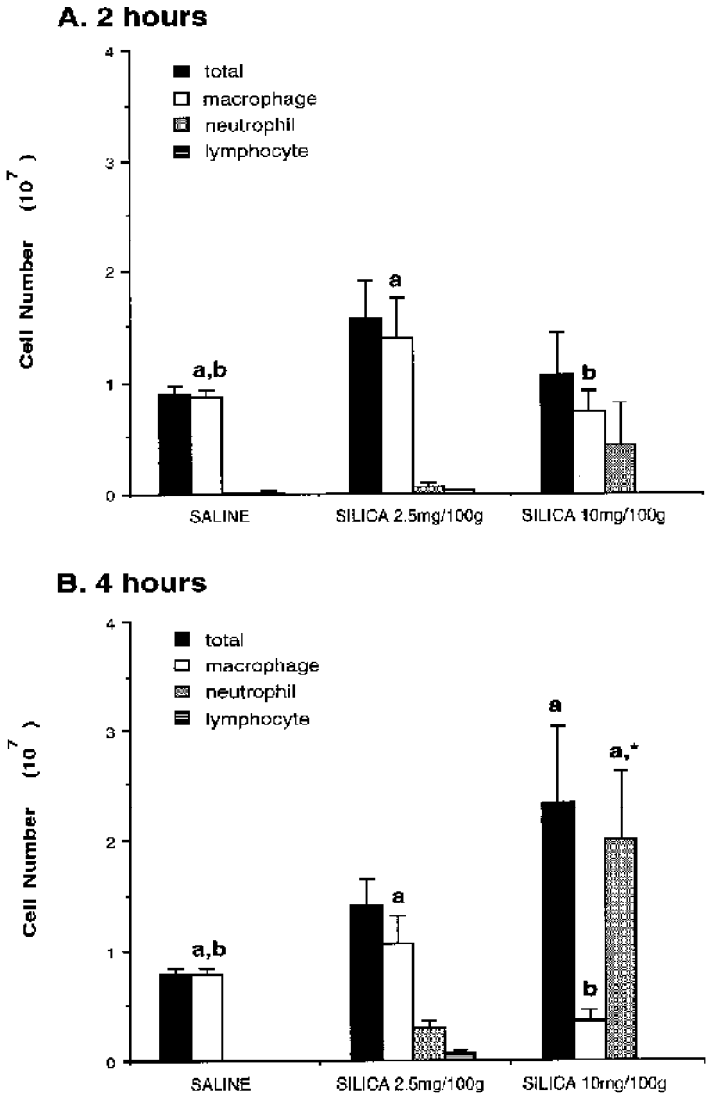

FIGURE 5. Mean BAL cell counts from animals (A) 2 h and (B) 4 h after saline (control) or silica instillation. Animals received a single intratracheal instillation of silica (2.5 or 10 mg/100 g body weight) or saline. Total cell number was obtained via a hemacytometer; alveolar macrophages, neutrophils, and lymphocytes were differentiated after staining with Wright Giemsa Sure Stain. Values are means ± SEM (n = 5–6). Letters (a, b) denote significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Within time or dose groups, different denotations represent significant differences from each other and the saline control.

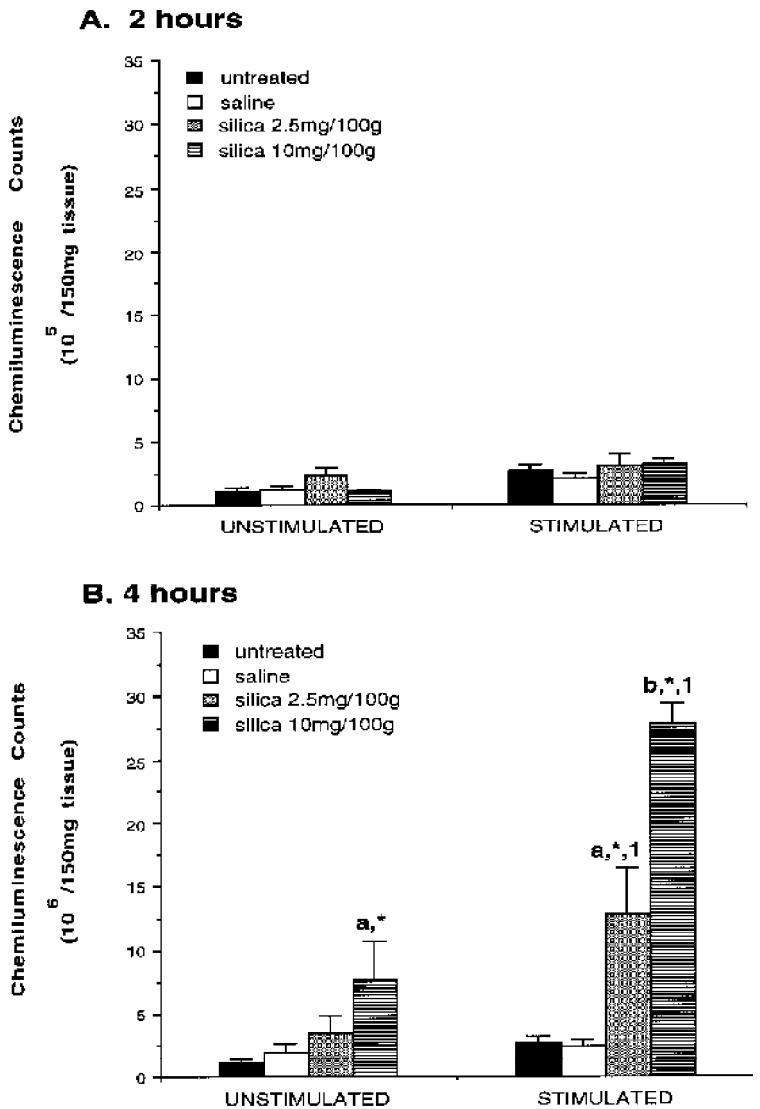

FIGURE 6. Luminol-dependent chemiluminescence (LDCL) generated by lung tissue from control (untreated and saline) and silica-treated rats at (A) 2 h and (B) 4 h. The two control groups establish baselines with respect to the silica-treated groups. LDCL was monitored in the presence of luminol (10^{-5} *M*) using a luminometer. LDCL was determined at rest (unstimulated) or after stimulation with 2×10^{-6} *M* phorbol myristate acetate (PMA). Values are means ± SEM ($n = 4$ for each time point). Letters (a, b) denote significant differences ($p < .05$) between groups within individual time points (i.e., saline vs. silica, 2.5 or 10 mg/100 g). Asterisk denotes significant differences ($p < .05$) in an individual treatment dose between time points (i.e., 2 h vs. 4 h). Number (1) denotes significant differences between unstimulated and PMA-stimulated within a specific group. Within time or dose groups, different denotations represent significant differences from each other and the saline control. *Note:* The same untreated group is used in both the 2- and 4-h graphs as a baseline for comparisons.

TABLE 1. Inhibition of PMA-Stimulated Luminol-Dependent Chemiluminescence (LDCL) Generated by Lung Issue from Control (Saline) and Silica-Treated Rats

Time	Group	PMA	Inhibitors[a]	
			PMA + SOD	PMA + L-NAME
2 h	Saline	2.144 ± 0.401[b]	0.475 ± 0.163 (77.8)[c]	0.750 ± 0.272 (65.0)
	Silica (2.5 mg/100 g)	3.030 ± 1.054	1.245 ± 0.586 (58.9)	1.444 ± 0.725 (52.3)
	Silica (10 mg/100 g)	3.104 ± 0.499	1.573 ± 0.969 (49.3)	1.037 ± 0.351 (66.6)
4 h	Saline	2.306 ± 0.587	0.625 ± 0.213 (72.9)	0.864 ± 0.514 (62.5)
	Silica (2.5 mg/100 g)	12.810 ± 3.641	4.326 ± 1.782 (66.2)	5.092 ± 2.191 (60.2)
	Silica (10 mg/100 g)	27.710 ± 1.826	7.451 ± 1.178 (73.1)	24.360 ± 3.203 (12.1)

[a]Superoxide dismutase (SOD, 0.2 mg/ml) or *N*-ω-nitro-L-arginine methyl ester (L-NAME, 1 m*M*) was added to the reaction mixture to determine involvement of superoxide anion and nitric oxide products, respectively.

[b]Values are total counts expressed as means ± SEM (10^5/150 mg tissue) (n = 3–4 for each time point).

[c]Percent inhibition compared to PMA-stimulated alone.

LDCL light was decreased in the 2.5 mg/100 g group by 52.3% (2 h) and 60.2% (4 h). The 10 mg/100 g group was decreased by 66.6% (2 h), but only exhibited a 12.1% reduction at 4 h. SOD and L-NAME also inhibited the background LDCL in saline-treated rats.

DISCUSSION

The present work was performed to investigate and characterize the responses that occur acutely within the lungs as a result of silica exposure. This is important because little information exists in the literature about the very early occurrences that initiate and propagate the later events of the toxic silica response. As a result of utilizing the most sensitive indicators of insoluble dust-induced bronchoalveolar toxicity (protein, β-glucuronidase, LDH, and neutrophils; Henderson, 1988), the data indicate that the toxic response due to silica begins as early as 2 h after exposure with significant increases of total protein in the BALF. This increase in total protein occurs before any increase in albumin can be detected, thus indicating that this detectable protein is originating from within the parenchymal tissue itself. The protein increase may be attributed to increased inflammatory mediator and chemotactic factor production and release by the resident alveolar macrophages

and/or cellular proteins being released after silica begins to exert its cytotoxic effects. Temporally, increased mediator/factor production appears likely, due to the resultant increase in neutrophil recruitment into the airspaces from the vasculature occurring at 4 h. The timing of this cellular appearance coincides with findings by Yuen et al. (1994). Utilizing a silica-induced pulmonary inflammation model, they found that neutrophils were recruited into the lung as early as 4 h after intratracheal (i.t.) instillation, and that neutrophil chemotactic activity could be detected directly in the BALF of these experimental animals. These results implicate the resident alveolar macrophage as playing a key role in the resulting inflammation.

After 4 h, multiple inflammatory/damage processes have occurred within the airspace of silica-instilled rats. The phagocytic cells of the lungs become activated or damaged due to the presence of silica, as evidenced by increased β-glucuronidase activity. These activated phagocytic cells release cytokines, enzymes, and inflammatory mediators in response to this challenge (Stein & Keshav, 1992). Increased levels of lactate dehydrogenase activity illustrate that cells present in the lungs are becoming damaged as a result of silica's cytotoxic properties and presumably by inflammatory mediators (cytokines and proteolytic enzymes) being released by cells involved in the inflammatory cascade. Vascular albumin also enters the alveoli as a result of direct damage to the alveolar–capillary barrier by silica itself and/or enhanced barrier permeability brought about by macrophage-derived mediators such as leukotriene B_4 (LTB_4) (Sprague et al., 1994; Yoshimura et al., 1994). This increase in vascular permeability also enables the neutrophils to enter the airspaces and compound the inflammatory processes.

In conjunction with the biochemical and cellular changes that are occurring, the phagocytic cells function in their normal capacity in trying to clear the lungs of the particulate matter. In doing so, a myriad of substances are secreted by these cells to aid in the overall process, including reactive oxygen and reactive nitrogen intermediates (Menzel, 1992; Castranova et al., 1993; Slade et al., 1993; Van Dyke et al., 1994). Chopped lung LDCL serves as a novel method of measuring the inflammatory and oxidative involvement of the "whole" organ (Antonini et al., 1994). In this manner it is possible to assess the activity of all inflammatory cells, those in the alveoli as well as those in the interstitium. It is recognized that this method has some limitations, primarily lung tissue acting as a physical barrier to added inhibitors, stimulants, and the emission of light, but the method nonetheless provides a unique approach for assessing the oxidative status of the inflamed lungs. By mixing tissue after chopping, responses of inflammatory cells that may be localized to specific regions of the lung are more uniformly assessed. Under nonstimulated conditions, the high dose of silica at 4 h was able to elicit a significant increase in LDCL. This finding is important in showing that silica alone can enhance the oxidative capacity of the lung tissue. In the present study, inhibition of the chopped lung LDCL by SOD and L-NAME implicates reactive oxygen and nitrogen species in playing a role in the acute phase pul-

monary response to silica. The diminished inhibitory ability of L-NAME in the 4-h high silica dose group may be due to the change in cell population (increased neutrophils) and the overwhelming release of nitrogen-based oxidants. The inhibitor L-NAME may not be in high enough concentration to suppress the LDCL to as great an extent.

It is known that the macrophage is capable of secreting pro-inflammatory, fibrogenic, and mitogenic cytokines, such as interleukin-1 (IL-1),IL-8, platelet-derived growth factor (PDGF), tumor necrosis factor α (TNF-α), etc. (Driscoll et al., 1991; Kovacs, 1991). Therefore, if one were able to employ a treatment modality early in the inflammatory cascade (macrophage processing and signaling, and cellular recruitment), then the late-phase sequelae might be attenuated or prevented. Along these lines, Pigeut et al. (1993) administered IL-1 receptor antagonist i.p. to mice instilled i.t. with silica and found a preventative or curative effect when hydroxyproline and histological preparations were assessed as indices for fibrosis. This finding is supportive of the idea that IL-1 and the related cytokine TNF-α have the ability to initiate the cascade of cytokines and other factors associated with the inflammatory responses (Vilcek & Lee, 1991; Mukaida et al., 1992). Hence, we see that intervention at the early stages in silica-induced pulmonary inflammation can prevent or reverse the common fibrotic endpoint.

Evidence exists that treatment with IL-1 receptor antagonist is effective even when administered after insult, as in the study by Leff and colleagues (1994). IL-1 was administered i.t. to rats to produce the insult; then, after 1.25–2.5 h had elapsed, IL-1 receptor antagonist was instilled. Results from this study suggest that IL-1 receptor antagonist treatment has the potential to decrease acute lung injury (lung leak, neutrophil influx, myeloperoxidase activity, and breath H_2O_2 concentration) even when given after the IL-1-inciting insult.

Silica-induced pulmonary damage is detectable as early as 2 h after instillation; this response develops into a definitive pulmonary inflammation at 4 h. Characterization of these early events is a necessary and logical step in the overall scheme of understanding and potentially treating silica-induced pulmonary inflammation.

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