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COMPARISON OF INDUCIBLE NITRIC OXIDE SYNTHASE GENE EXPRESSION AND LUNG INFLAMMATION FOLLOWING INTRATRACHEAL INSTILLATION OF SILICA, COAL, CARBONYL IRON, OR TITANIUM DIOXIDE IN RATS

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The pulmonary toxicity of the respirable dusts silica, coal, carbonyl iron, and titanium dioxide on alveolar macrophage (AM) and neutrophil (PMN) inducible nitric oxide synthase (iNOS) gene expression and nitric oxide (NO) production was investigated. Rats were intratracheally instilled with 5 mg/100 g body weight of silica, coal, carbonyl iron, or titanium dioxide. The dust particles averaged less than 5 μm in diameter. Bronchoalveolar lavage was performed 24 h later. Bronchoalveolar lavage cell (BALC) differentials, iNOS gene expression and NO production by BALC (measured indirectly as NO-dependent chemiluminescence), and lavageable lung protein levels were measured. Analyzed on an equal mass basis, silica, coal, and titanium dioxide dusts increased the production of iNOS-dependent NO by AM. Silica and titanium dioxide both increased the levels of iNOS mRNA while carbonyl iron and coal did not. Each dust caused an increase in PMN, indicating an inflammatory response. Carbonyl iron and titanium dioxide decreased the numbers of AM. Levels of acellular lavageable lung protein were increased by silica, carbonyl iron, and titanium dioxide. When exposure was normalized for an equal number of particles, the pneumotoxic dusts, silica and coal, caused more inflammation and NO production than the nuisance dusts, carbonyl iron and titanium dioxide. Therefore, it appears

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that particle number is a more appropriate metric of exposure than mass when comparing the relative pathogenicity of dusts of different sizes. Furthermore, since the potency of these dusts (on a particle number basis) to increase iNOS gene expression reflects their inflammatory and pathogenic potential, it is proposed that NO may contribute to the early inflammatory damage observed in the lung following dust exposure.

Silica has long been known to be highly pneumotoxic and fibrogenic in humans (Peters, 1986). Coal dust causes pneumoconiosis but is considered less fibrogenic than silica (Parkes, 1994). Dust standards in the workplace set at 0.1 mg/m^3 for silica and 2 mg/m^3 for coal reflect the relative toxicity of these two dusts. The nuisance dusts carbonyl iron and titanium dioxide are considered even less toxic, having exposure limits of 5 mg/m^3. Animal studies demonstrate a prolonged inflammatory response to silica leading to silicosis (Bowden & Adamson, 1984; Driscoll et al., 1990, 1991; Lindenschmidt et al., 1990; Warheit et al., 1991). Coal dust activates alveolar macrophages (AM), but does not lead to fibrosis (Bowden & Adamson, 1978; Castranova et al., 1985; King et al., 1958; Martin et al., 1977). The nuisance dusts titanium dioxide (Driscoll et al., 1990, 1991; Lindenschmidt et al., 1990) and carbonyl iron (Warheit et al., 1991) cause inflammation of a short duration, with little lung damage, that does not usually lead to fibrosis. Silica, compared to iron, causes greater damage to the blood–air barrier of the lung and a greater recruitment of polymorphonuclear leukocytes (PMN) (Beck et al., 1982). These observations show that silica is often more toxic than coal, titanium dioxide, or iron in animal models. However, coal is generally more toxic than carbonyl iron or titanium dioxide, both of which have been used as nontoxic control dusts in many studies.

Several studies have clearly established the potential of AM and PMN to produce nitric oxide (NO) when exposed to inflammatory stimuli. Inducible nitric oxide synthase (iNOS) gene expression in AM and PMN is upregulated by specific stimuli. Nitrite and nitrate production in rat AM is increased by the addition of bacterial lipopolysaccharide (LPS), interferon-γ (IFN-γ) (Stuehr & Marletta, 1985; Hibbs et al., 1987), and opsonized zymosan (Jorens et al., 1991). Enhanced NO production has been reported in rat PMN in response to LPS (Yui et al., 1991) and in human PMN in response to platelet aggregation (Salvemini et al., 1989) or phorbol 12-myristate 13-acetate (Goode et al., 1994). The anticancer agent bleomycin, known to cause pulmonary inflammation, increases levels of nitrite produced by rat AM (Huot & Hacker, 1990). The intratracheal instillation of silica increases *N*-ω-nitro-L-arginine methyl ester (L-NAME) inhibitable chemiluminescence in AM and upregulates iNOS gene expression in rat AM and PMN (Blackford et al., 1994). Rat AM also produce the iNOS protein as well as NO after inhalation of ozone (Pendino et al., 1993).

NO may play important roles in mediating inflammatory injury,

possibly by combining with superoxide to form peroxynitrite (Blough & Zafiriou, 1985; Van Dyke et al., 1994). PMN production of NO, and the subsequent formation of peroxynitrite, has been postulated to damage the blood–air barrier following deposition of immune complexes (Mulligan et al., 1991). NO has also been found to directly stimulate cyclooxygenase enzymes, resulting in the formation of the proinflammatory prostaglandin E (Salvemini et al., 1993), linking NO to the inflammatory response.

Since NO production has been linked to inflammation and lung damage, the goal of this study was to characterize and compare changes in AM and PMN iNOS gene expression after exposure to silica, coal, titanium dioxide, or carbonyl iron. The data were analyzed to determine if induction of iNOS gene expression by these dusts is related to their ability to cause lung damage and disease.

MATERIALS AND METHODS

Experimental Design

The experimental design consisted of rats intratracheally instilled with either saline, silica, coal, carbonyl iron, or titanium dioxide. After 24 h, the rats were killed and bronchoalveolar lavage (BAL) fluid containing cells and protein was collected from both treated rats and matching controls. The total numbers of cells and the population differentials were determined. Total lavageable lung protein was measured. iNOS messenger RNA levels in BALC were measured using Northern analysis, and NO production by AM was determined as NO-dependent chemiluminescence.

Animals

Specific-pathogen-free male Fischer 344 rats (175–225 g) were obtained from Hilltop Labs (Scottdale, PA). The rats were kept in cages covered with HEPA filters and provided with rat feed and water ad libitum.

Intratracheal Instillation

The rats were lightly anesthetized with an intraperitoneal injection of 0.5 ml of a 1% solution of sodium methohexital (Brevitol, Eli Lilly Co., Indianapolis, IN). Using the method of Brain et al. (1976), rats were intratracheally instilled with dust particles suspended in 0.5 ml of saline using a 20-gauge, 4-in ball-tipped animal feeding needle (Perfectum, New Hyde Park, NY). Each rat received 5 mg/100 g body weight of dust particles. Rats intratracheally instilled with 0.5 ml saline vehicle served as controls.

As a positive indication that each rat received dust in the lungs, cytospin slides were examined from each animal. The slides were

examined under light microscopy to identify dust particles in association with AM and PMN, an indication that the dust particles had reached the alveoli of the lung.

Particle Characterization

Coal mine dust was obtained from Blacksville, WV, and crystalline silica (Min-U-Sil) was obtained from U.S. Silica Corporation (Berkeley Springs, WV). Carbonyl iron and titanium dioxide were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Particle characterization was determined microscopically with a Jeol JSM-6400 scanning electron microscope. Particles were prepared for examination by first making 1-mg/ml hydrosols for each of the dusts. Particles were then dispersed using a Heat Systems ultrasonic processor. Various dilutions were made and known volumes were filtered through polycarbonate filters. The filter preparations were gold/palladium

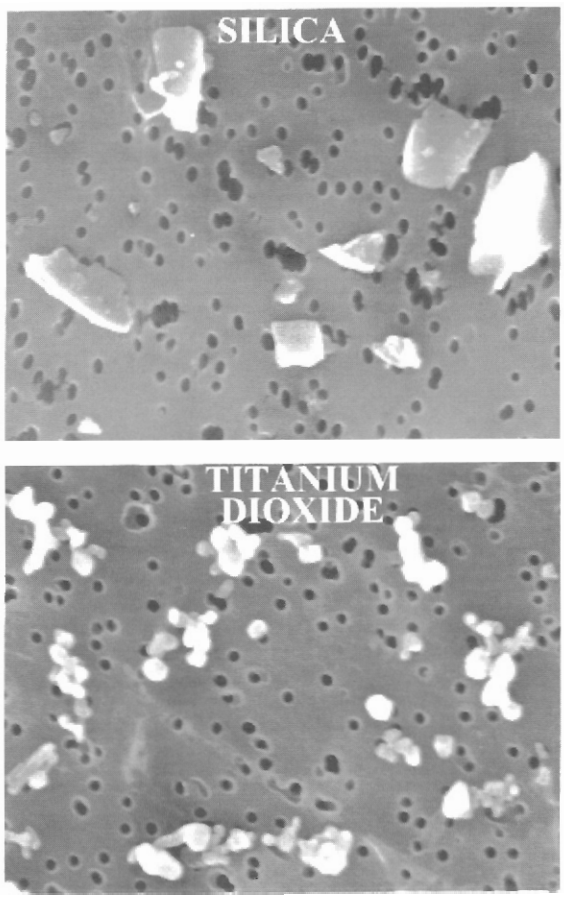

FIGURE 1. Scanning electron micrographs of silica and titanium dioxide dusts.

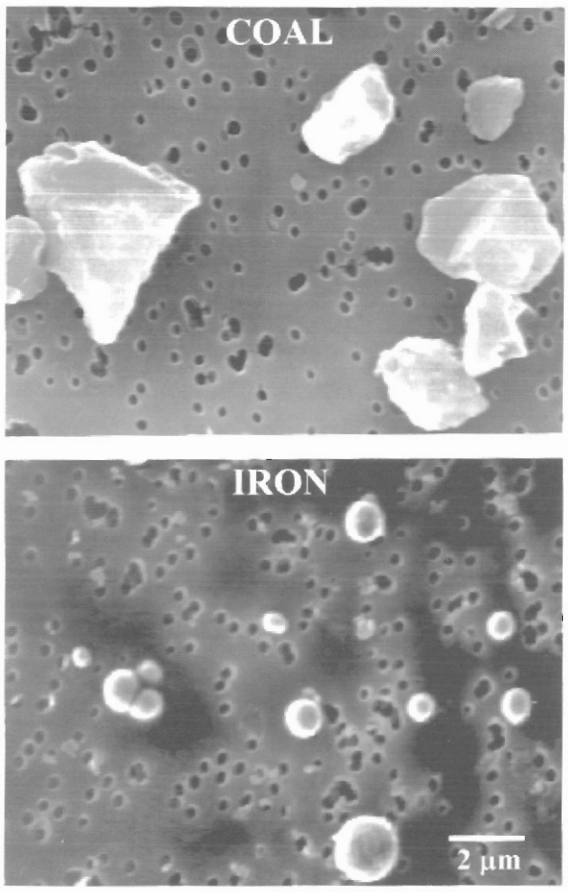

FIGURE 1. *(Continued)* Scanning electron micrographs of coal and carbonyl iron dusts.

coated and examined at magnifications ranging from 100 to 40,000× at an accelerating voltage of 20 kV (Figure 1). Particle size and count measurements were made directly from the microscope screen. Circular equivalent area diameters of the dust samples were recorded (Figure 2). Cumulative size data showed that 95% of the coal mine dust particles measured less than 6 µm in equivalent area diameter (medium diameter ≈ 1.9 µm). For silica particles, 95% were less than 4 µm (median diameter ≈ 0.9 µm), while 95% of the carbonyl iron particles were less than 2 µm (median diameter ≈ 0.6 µm), and 95% of the titanium dioxide particles were less than 1 µm (median diameter ≈ 0.5 µm).

The smallest particle, titanium dioxide, had the highest number of particles per mass with 1.2×10^{12} particles/g (Figure 3). Carbonyl iron had 2.5×10^{11} particles/g, silica had 1.7×10^{11} particles/g, and coal dust had 4.5×10^{10} particles/g.

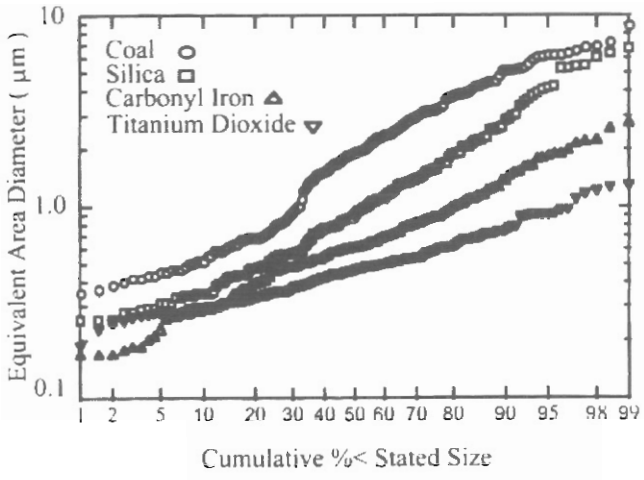

FIGURE 2. Cumulative percentage of less than stated size of silica, coal, carbonyl iron, and titanium dioxide dusts measured in equivalent area diameter (μm).

Isolation of BALC

Twenty-four hours after treatment the rats were anesthetized with 0.2 g/kg body weight of sodium pentobarbital and exsanguinated by cutting the left renal artery. Bronchoalveolar lavage was performed through a tracheal cannula with 10 pulmonary lavages of 8 ml each using ice-cold Ca^{2+},Mg^{2+}-free phosphate-buffered medium (145 m*M* NaCl, 5 m*M* KCl, 1.9 m*M* NaH_2PO_4, 9.35 m*M* Na_2HPO_4, and 5.5 m*M* dextrose; pH 7.4). The bronchoalveolar lavagate was centrifuged (500 × g for 10 min) and the bronchoalveolar lavage cells (BALC) were resuspended, centrifuged, and resuspended again in HEPES-

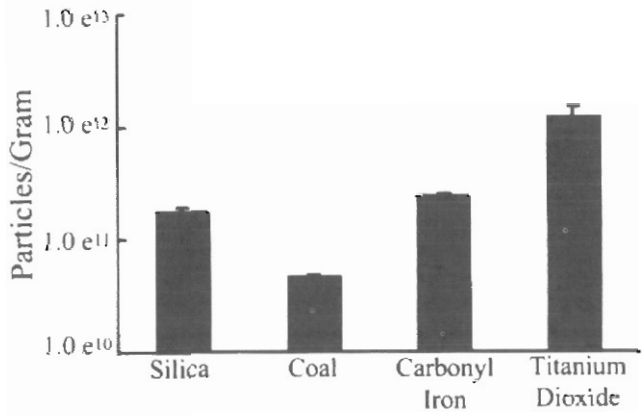

FIGURE 3. Particle counts for silica, coal, carbonyl iron, and titanium dioxide dusts measured in particles per gram.

buffered (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) medium (145 m*M* NaCl, 5 m*M* KCl, 10 m*M* HEPES, 5.5 m*M* dextrose, and 1.0 m*M* $CaCl_2$; pH 7.4).

RNA Isolation

BALC were isolated from the lavagate of each rat by centrifugation (500 × g for 10 min). The BALC were washed with Ca^{2+},Mg^{2+}-free phosphate-buffered medium and centrifuged (500 × g for 10 min). Total cellular RNA was extracted by a modification of the acidic guanidinium thiocyanate–phenol–chloroform method (Chomczynski & Sacchi, 1987). All solutions used for Northern analysis were prepared with 1% diethyl pyrocarbonate (DEPC; Sigma, St. Louis, MO) treated, double-distilled water. The BALC pellet was lysed in 5 ml of 4 *M* guanidinium thiocyanate, 0.5% Sarcosyl (Sigma), 5 ml of water-saturated phenol (Sigma), 1 ml chloroform (Sigma), and 0.5 ml of 2 *M* sodium acetate. The solution was centrifuged (2800 × g for 30 min). The aqueous phase was transferred to a clean tube and an equal volume of isopropanol (Sigma) was added and stored at –20°C overnight. The tubes were centrifuged, the solution was removed, and the RNA pellet was resuspended in an equal volume of 80% ethanol and centrifuged. Ethanol was decanted off; the RNA pellet was vacuum dried for 10 min, and solubilized in 0.5% sodium dodecyl sulfate (SDS). The RNA was quantitated in a spectrophotometer using the extinction coefficient of 260λ.

Hybridization Probe Generation

The plasmid CL-BS containing a 4100-bp cDNA fragment for mouse iNOS was kindly provided by Dr. S. H. Snyder (Johns Hopkins University, Baltimore, MD) (Lowenstein et al., 1992). DNA templates were amplified by conventional polymerase chain reaction (PCR) using a GeneAmp DNA amplification reagent kit (Perkin-Elmer/Cetus, Norwalk, CT) and 20-mer synthetic oligonucleotide primers. The iNOS sense primer sequence was 5'-TGACCATCGTTGACCACCAC (pos 2655) and the anti-sense primer sequence was 5'-CTCCCCTCCCAGTTCCTCCA (pos 3474) to create a single-stranded DNA (ssDNA) probe of 819 bp. Very high efficiency ssDNA hybridization probes were generated by PCR (Konat et al., 1991) on these templates using anti-sense primer and ^{32}P-α-labeled dCTP (ICN Biochemicals, Costa Mesta, CA). Probes were purified on a G-25 quick spin column (Boehringer, Indianapolis, IN).

Northern Blot Analysis

Northern analysis was performed as described by Ye et al. (1992). Briefly, 20 μg of total RNA was diluted in loading buffer [3-(*N*-morpholino)propane-sulfonic acid (MOPS), 40% formamide and 15%

formaldehyde; Sigma]. The RNA was electrophoresed on a 5.6% formaldehyde denaturing 1% agarose gel and subsequently vacuum transferred onto a Nytran membrane (Schleicher and Schuell, Keene, NH). The membrane was washed with 2× sodium chloride/sodium citrate (SSC), dried at 65°C, and cross-linked using ultraviolet (UV) light. The membrane was stained with 0.02% methylene blue in 50 m*M* sodium acetate buffer (pH 4.8) to visualize ribosomal RNA.

The membrane was washed for 30 min with 50 ml 2 × SSC/0.1% SDS at 60°C and prehybridized at 68°C for 30 min with 4 ml Quickhyb hybridization buffer (Stratagene) plus 200 μl of 10 mg/ml herring sperm DNA (Promega, Madison, WI). Herring sperm was boiled for 10 min before addition. Subsequently, an ssDNA antisense iNOS probe (5×10^7 cpm) was added, and the hybridization was performed for 1 h at 68°C. The membrane was washed 3 times with 50 ml 2× SSC/0.1% SDS at room temperature, washed again for 30 min with 50 ml 0.2× SSC/0.1% SDS at 60°C, and finally opposed to Kodak XAR film with one intensifying screen and exposed at –80°C for various periods of time to ensure linearity.

Image Analysis

Images of the methylene blue stained membranes were captured by a PC-based Optimas imaging system (Bioscan, Edmonds, WA) to establish optical density for the ribosomal bands, thus assessing the total amount of RNA transferred onto the membrane. The use of ribosomal RNA to normalize Northern blots has been established using oligonucleotide probes (Barbu & Dautry, 1989). The optical density of methylene blue-stained rRNA bands was used to normalize loading of total RNA onto the membranes. The optical density was also determined for each iNOS mRNA autoradiographic signal. The optical density for each autoradiographic signal was divided by the corresponding optical density for rRNA to normalize the autoradiographic signal.

AM Chemiluminescence Assay

Chemiluminescence (cpm/0.5×10^6 AM/10 min) generated by AM from individual rats was measured using a modification of the procedure of Van Dyke et al. (1977). BALC were resuspended in HEPES-buffered medium at a final concentration of 1×10^6 AM/ml. From each cell suspension, 0.5×10^6 AM were used to determine chemiluminescence under 4 separate conditions: (1) resting cells, with no treatment, to serve as a baseline control; (2) resting cells pretreated 10 min with 1 m*M* *N*-ω-nitro-L-arginine methyl ester (L-NAME, Sigma) to determine baseline L-NAME-inhibitable chemiluminescence; (3) cells treated with 2 mg/ml unopsonized zymosan (Sigma) to stimulate chemiluminescence in AM; and (4) BALC treated with 2 mg/ml unopsonized zymosan and 1 m*M* L-NAME to determine the percentage of

iNOS-dependent (L-NAME-inhibitable) chemiluminescence in AM following stimulation with unopsonized zymosan. All tubes were incubated for 10 min at 37°C in a shaking water bath prior to chemiluminescence determination. The cell suspensions were transferred into polystyrene luminometer tubes and 0.15 ml luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was added to a final concentration of 8 mg% of luminol (Sigma). Chemiluminescence was measured using a Berthold LB953 luminometer (Wallac, Inc., Gaithersburg, MD) at 390–620 nm for 10 min. The integral of counts per minute (cpm) versus time was determined and used to compare the total chemiluminescence between samples. Zymosan-induced total chemiluminescence was calculated as the response to zymosan minus the resting level of chemiluminescence. iNOS-dependent chemiluminescence was determined by subtracting zymosan-dependent total chemiluminescence in the presence of the iNOS inhibitor, L-NAME, from samples without inhibitor. Since PMN do not respond to unopsonized zymosan, this iNOS-dependent chemiluminescence represents NO production from AM (Castranova et al., 1990).

Cell Differentials

Cell counts and differentials were determined using an electronic cell counter equipped with a cell sizing attachment (Coulter model ZBI with a Channelizer 256, Coulter Electronics, Inc., Hialeah, FL) as described by Lane and Mehta (1990). Lymphocytes, PMN, and AM were identified by their distinctive cell volumes (Castranova et al., 1990).

Lavageable Lung Protein Assay

A protein assay (Bio-Rad, Hercules, CA) based on the Bradford reaction was used to quantify the amount of lavageable lung protein in the lung following exposure to particles (Bowers-Komro et al., 1989). Bronchoalveolar lavage fluid (10 µl) from the first 5 ml of bronchoalveolar lavage, centrifuged free of cells, was added to a 96-well titer plate. Coomasssie blue dye (200 µl) was added to each well and then mixed. The plate was incubated at room temperature for 5 min. The absorbance was measured using a spectrophotometer at 595λ and compared to a standard curve to determine the protein content of each sample.

Statistical Analysis

Mean values of the inverse log of the integrated grey value (ILIGV) of iNOS autoradiograph signals, iNOS-dependent chemiluminescence, and cellular differentials were compared by one-way analysis of variance (ANOVA) using the Macintosh-based software Statview II (Abacus Concepts, Berkeley, CA). Significance was set at $p \leq .05$.

When significant F values were obtained, individual means were compared using the least significant difference test.

RESULTS

iNOS Message

BALC iNOS mRNA steady-state levels were increased 24 h after silica or titanium dioxide instillation relative to saline (Figure 4). Silica increased the steady-state level of BALC iNOS mRNA by 4.7-fold and titanium dioxide caused a 4.3-fold increase. BALC iNOS mRNA steady-state levels were not significantly elevated after intratracheal instillation of coal or carbonyl iron relative to saline.

Chemiluminescence

iNOS-dependent chemiluminescence was significantly increased after intratracheal instillation of silica, coal, or titanium dioxide compared to the combined saline control group (Figure 5). In response to silica exposure, AM iNOS-dependent chemiluminescence was increased by 25.7-fold and comprised approximately 37% of the total chemiluminescence. Following exposure to coal dust, AM iNOS-dependent chemiluminescence increased by 11.3-fold and comprised approximately 41% of the total chemiluminescence. Following exposure to titanium dioxide, iNOS-dependent chemiluminescence increased by 6.7-fold and comprised approximately 40% of the total chemiluminescence. Following exposure to carbonyl iron, iNOS-dependent chemiluminescence increased by 2.9-fold and comprised approximately 35% of the total chemiluminescence.

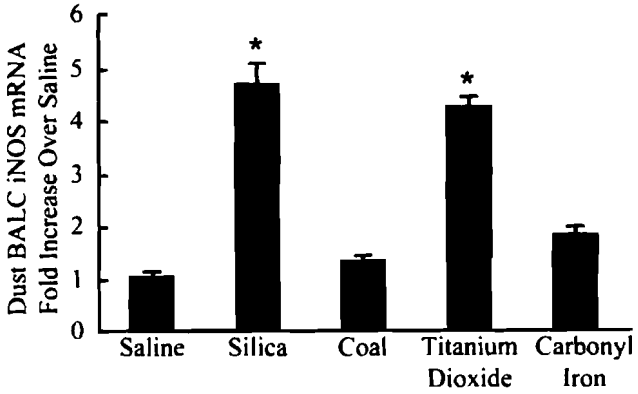

FIGURE 4. BALC iNOS mRNA levels expressed as fold increases over saline. The values (mean ± SEM) are normalized for total RNA loaded in each lane as described in Methods. Asterisk indicates significant difference compared to saline, $p \leq .05$.

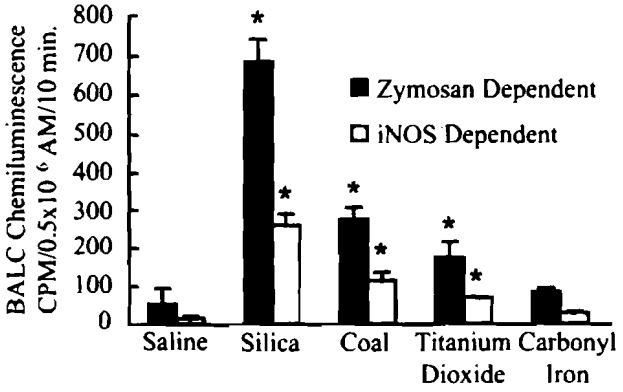

FIGURE 5. Zymosan-stimulated and iNOS-dependent zymosan-stimulated AM chemiluminescence from rats treated with saline (average from all treatments), silica, coal, titanium dioxide, and carbonyl iron (mean ± SEM). Asterisk indicates significant difference compared to saline, $p \leq .05$.

Cellular Differentials

BAL cellular differential analysis 24 h following dust treatment is shown in Figure 6. No significant changes in AM yield were noted due to silica or coal treatment, while significant decreases were observed in AM following carbonyl iron treatment (↓35%) and after titanium dioxide treatment (↓58%). The PMN population was significantly increased due to silica (↑1569%), titanium dioxide treatment (↑166%), coal (↑145%), or carbonyl iron (↑97%) treatment compared to saline. The lymphocyte population did not change due to any dust treatment.

Lavageable Lung Protein

Values for lavageable lung protein in the first 5 ml of acellular BAL fluid from rats intratracheally instilled with silica, coal, titanium

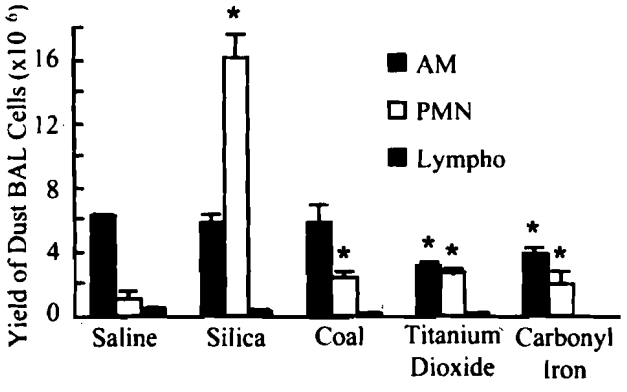

FIGURE 6. BALC differential analysis of AM, PMN, and lymphocytes expressed as millions of cells (mean ± SEM). Asterisk indicates significant difference compared to saline, $p \leq .05$.

dioxide, or carbonyl iron are given in Figure 7. Acellular BAL protein was elevated by 365%, 213%, 168%, or 89% 24 h after exposure to silica, carbonyl iron, titanium dioxide, or coal dust, respectively.

DISCUSSION

The experimental design of the present investigation was to expose rats by intratracheal instillation to an equal mass (5 mg/g body weight) of various particulates. The objective was to employ dusts documented to exhibit a broad range of pathogenicity in order to test the hypothesis that NO production was related to pulmonary toxicity. Silica and coal dust were chosen because they cause pneumoconiosis in both animals and humans, with silica being the more fibrogenic dust (Lapp & Castranova, 1993). In contrast, carbonyl iron and titanium dioxide have been reported to be relatively inert in animal models (Driscoll et al., 1990, 1991; Warheit et al., 1991). Likewise, Chen and Fayerweather (1988) reported that pulmonary disease in workers exposed to titanium dioxide did not differ from controls. The results of the present study indicate that, analyzed on an equal mass basis, all the dusts tested caused some degree of pulmonary inflammation and damage to the alveolar air–blood barrier 24 h after intratracheal instillation. The sequence for inflammatory potency of the dusts, measured as lavageable PMN, was silica followed by titanium dioxide, coal, and carbonyl iron. Toxicity sequence, measured as acellular lavage protein, was silica followed by carbonyl iron, titanium dioxide, and then coal dust. The data indicate that silica, coal dust and titanium dioxide significantly elevated NO production by BALC, while mRNA for iNOS in BALC was significantly increased 24 h following instillation of silica or titanium dioxide.

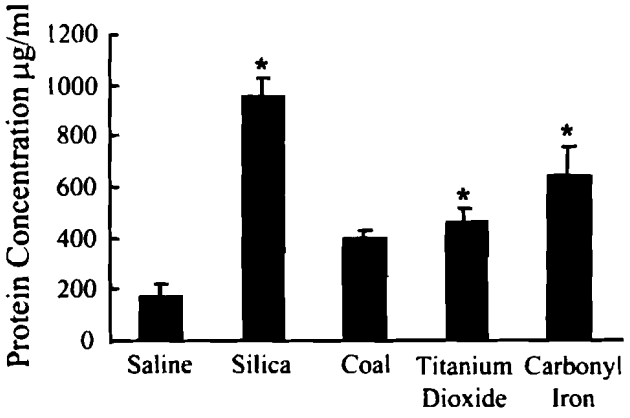

FIGURE 7. Total acellular protein in the first lavage from rats instilled with saline or different dusts expressed as µg/ml (mean ± SEM). Asterisk indicates significant difference compared to saline, $p \leq .05$.

TABLE 1. Normalized responses to dusts

Dust	iNOS message	NO-dependent CL	PMN	Protein
Silica	26.55	179.41	115.57	24.38
Coal	8.56	283.57	41.14	21.26
Carbonyl iron	4.00	9.30	5.00	9.70
Titanium dioxide	3.26	5.87	1.71	1.07

Note. Rats were exposed by intratracheal instillation to dust particles (5 mg/100 g body weight) and bronchoalveolar lavage performed 24 h postexposure. Values for BALC iNOS mRNA, L-NAME inhibitable zymosan-stimulated chemiluminescence, PMN yield, and acellular lavage protein were normalized for exposure to an equal number of particles for each dust (particle count data taken from Figure 3).

These data indicate that silica is the most potent inducer of mRNA for iNOS and NO release. It also causes the greatest pulmonary inflammation and damage. However, the results fail to demonstrate a clear correlation between induction of NO release or iNOS message and indicators of pulmonary inflammation or damage for the other dusts. In addition, there was not always agreement between reported pneumoconiotic potential and the ability to cause inflammation or induce iNOS message. Indeed, titanium dioxide, a so-called nuisance dust, induced more mRNA for iNOS and caused a greater infiltration of PMN than coal dust did.

Failure of the data to correlate well with the known pneumoconiotic potency of these dusts led us to reevaluate the experimental design, that is, the comparison of the dust exposures on an equal mass basis. Data from our laboratory suggest that analysis of toxicity on an equal particle count basis may be more appropriate (Castranova et al., 1994). As indicated in Figure 2, the dusts used differ greatly in mean diameter. This resulted in widely different particle counts per mass among these dusts. For example, there were approximately 27 times as many particles of titanium dioxide than coal dust per given mass (Figure 3).

Considering these issues, the data were reanalyzed by normalizing the responses to exposure to an equal number of particles for each dust, by dividing the responses by the relative number of particles per mass. The results of the analysis are given in Table 1. This analysis indicates that the sequence of inflammatory and toxic potency is silica > coal dust > carbonyl iron > titanium dioxide. This sequence correlates well with the known pathogenic potential of these dusts. In addition, the pathogenic dusts, silica and coal, exhibit a greater enhancement of the nitric oxide system than the so-called nuisance dusts, titanium dioxide and carbonyl iron. Therefore, a strong relationship is demonstrated between induction of iNOS message and NO release and particle-induced pulmonary inflammation and lung damage. NO is known to be capable of generating the highly potent oxidant

peroxynitrite (Blough & Zafiriou, 1985; Ischiropoulous et al., 1992). Peroxynitrite has been proposed to mediate damage to the lung parenchyma (Beckman et al., 1990; Mulligan et al., 1991) and thus could result in the protein leak reported in the present investigation. Nitric oxide has also been shown to increase the release of pro-inflammatory metabolites of arachidonic acid (Salvemini et al., 1993). This could be related to the infiltration of PMN seen in the present study.

In conclusion, the present investigation indicates that pulmonary reactions to particulate exposure should be evaluated on both a particle mass and particle count basis, since conclusions derived from these analyses often differ. The data suggest that when analyzed on an equivalent particle count basis a strong correlation exists between induction of iNOS message, NO production by pulmonary phagocytes, and adverse pulmonary reactions to a variety of dusts with reportedly differing toxicity. This lung damage may result in part from an elevated oxidant burden mediated by peroxynitrite generated from activated phagocytes.

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