Agents Actions 41: 44–49 (1994)

0065-4299/94/020044-06 $ 1.50+0.20/0

The inhibition of silica-induced lung inflammation by dexamethasone as measured by bronchoalveolar lavage fluid parameters and peroxynitrite-dependent chemiluminescence

Knox Van Dyke, James M. Antonini, Lixin Wu, Zuguang Ye, and Mark J. Reasor

Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center of West Virginia University, Morgantown, WV 26506, USA

Received 22 July 1993; accepted by E. R. Pettipher 8 November 1993

Abstract. The inhalation of silica has been shown to produce a dramatic inflammatory and toxic response within the lungs of humans and laboratory animals. Currently, no effective treatment exists for workers who may have been exposed to the inhalation of silica. The objective of this study was to develop an animal model in which we could evaluate the effect that anti-inflammatory steroids have on the acute silica-induced pulmonary inflammatory response. Male Fischer 344 rats were pretreated with either dexamethasone (2 mg/kg) or saline vehicle (i.p.) on days 1, 3, and 5. On day 6, the animals from the two groups were then intratracheally instilled with either silica (20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). Twenty-four hours after the instillations in the non-steroid group, significant increases occurred in total protein, total number of cells, neutrophils, and lymphocytes recovered from the lungs of animals treated with silica compared to saline controls. Silica also caused dramatic increases in the luminol-dependent chemiluminescence (LDCL) of lung tissue and bronchoalveolar lavage (BAL) cells. The LDCL reaction was markedly decreased by either superoxide dismutase (SOD) or *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME). SOD is involved in the enzymatic breakdown of superoxide anion, while L-NAME, a nitric oxide (NO) synthase inhibitor, prevents the formation of NO. When the superoxide anion and NO react, they form the highly oxidizing substance peroxynitrite. This study then implicates peroxynitrite as an agent which may be involved in the silica-induced oxidant lung injury. When the animals were pretreated with the steroid dexamethasone, there was a complete protection against the biochemical, cellular, and chemiluminescent indices of damage caused by silica. The mechanism in which the steroid protects the lung from damage may be due to the ability of dexamethasone to block the induction of NO synthase. With further study in animals, the anti-inflammatory steroids may be useful in the treatment of silica-induced lung injury.

Key words: Silica – Lung – Inflammation – Dexamethasone – Peroxynitrite – Chemiluminescence

Correspondence to: K. Van Dyke

Introduction

In the early 1930s, the public became aware of the surprising toxicity of silica. In the infamous "Hawk's Nest Incident", hundreds of workers in West Virginia died within months of inhaling highly concentrated silica dust. This airborne toxicity resulted from drilling a hydroelectric tunnel with dull drills. The incident has been termed the greatest industrial accident in the history of the United States [1]. Similar toxicity occurs worldwide where miners inhale small particles of silica (0.5 μm diameter or less). Many workers encounter silica from various industrial applications and are possible candidates for the toxicity of silica [2].

Exposure of the lungs to silica particles results in damage to the respiratory epithelium and interstitial matrix [3]. The cytotoxic activity of silica appears related to the rupture of the lysosomal membrane of the alveolar macrophage and the release of lysosomal enzymes into the cytoplasm. Following lysis of the macrophage, the free silica particles are once again released to be ingested by fresh macrophages, some of which are in turn also killed [4]. Thus, unlike most other particulates, silica is not detoxified by macrophage phagocytosis. Eventually the lung attempts to localize the damage by walling off the toxic silica by producing scarring or fibrosis of the lung. During fibrosis, the alveolar wall is thickened, preventing or inhibiting the exchange of oxygen across the alveoli and into the blood of the lung [5].

However, before fibrosis can occur a tremendous inflammation begins. In fact, in rats inflammation occurs within 24 h of instilling or breathing silica into the lungs, but the first signs of fibrosis do not occur until two months

later [6]. It is most likely the two events are linked and, therefore, it follows that the prevention of fibrosis might occur if the inflammation could be stopped.

To develop a strategy to treat the silica-induced inflammation, one would have to understand how the damage is manifested. In a previous study, we found that the cytotoxicity of silica is linked to a large increase of calcium uptake into phagocytic cells as a result of contact with silica [7]. If an esterified calcium chelator (INDO-1 AM) is preloaded into the phagocytic cell, it protects against the toxicity caused by silica. Certainly, excessive calcium placed inside a cell could prove deadly to the cell because the cell regulates its internal concentration very tightly using gate-regulated channels [8]. There is evidence that increases in intracellular calcium can activate the entire inflammatory cascade which includes interleukins, growth factors, leukotriene production, nitric oxide (NO) synthesis and oxidative burst production of superoxide anion $[\cdot O_2]^{(-)}$ [9–13].

NO synthase is the enzyme responsible for the generation of NO. This enzyme exists in two forms, i.e., a constitutive form present in endothelial cells and brain [14] and an inducible form found in macrophages [15]. In pulmonary phagocytes, the activity of the constitutive form of NO synthase is low. However, the enzyme can be induced in macrophages by silica (unpublished results). Once generated, NO can combine with superoxide anion to form peroxynitrite ($OONO^-$). Peroxynitrite possesses 1000 times the oxidative ability of hydrogen peroxide [16]. Peroxynitrite has also been shown to oxidize sulfhydryl groups and to inactivate alpha-1-proteinase inhibitor, making the lung more susceptible to damage caused by proteolytic enzymes [17]. It is reasonable to believe that phagocytic cells activated by silica may produce large amounts of peroxynitrite, which in turn may lead to oxidant lung injury.

Steroids have been shown to block the induction of NO synthase [18]. Therefore, it seems logical to treat the pulmonary toxicity caused by the exposure of silica by inhibiting the induction of NO synthase and thus preventing the formation of peroxynitrite. One objective of this study then was to develop an animal model by using an anti-inflammatory steroid in an attempt to reduce the silica-induced pneumotoxic response. For our investigation, dexamethasone, a potent anti-inflammatory steroid, was administered to rats before the intratracheal instillation of silica to inhibit the induction of NO synthase and therefore block the production of the potent oxidant, peroxynitrite.

In the assessment of lung damage for this study, a variety of cellular and biochemical indices of pneumotoxicity were utilized. In conjunction with the measurement of total protein of the acellular lavage fluid and the determination of the cellular differentials, the luminol-dependent chemiluminescence (LDCL) of the cells and lung tissue recovered from exposed animals was also measured. LDCL, the emission of light, is accompanied by the release of reactive forms of oxygen from phagocytic cells when they are stimulated. With the use of LDCL, another goal of this study then was to investigate the role of peroxynitrite in the process of silica-induced cytotoxicity.

Materials and methods

Chemicals and reagents

The silica (alpha-quartz) particles were originally from Pennsylvania Sand and Glass Co., Pittsburgh, Pennsylvania. The size of the particles was 5 μm or less, with a silica content of 98.05%. Dexamethasone sodium phosphate was purchased from American Regent Laboratories, Shirley, NY. Bovine serum albumin was from Miles Lab. Inc., Kankakee, IL. HEPES, superoxide dismutase (SOD), *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME) and phorbol myristate acetate (PMA) were obtained from Sigma Chemical Co., St. Louis, MO. Hanks' buffer was acquired from Gibco Lab., Life Technologies, Inc., Grand Island, NY. Sodium pentobarbital was obtained from The Butler Company, Columbus, OH. Sodium methohexital was obtained from Eli Lilly & Co., Indianapolis, IN. Wright-Giemsa stain was ordered from Fisher Scientific Pittsburgh, PA.

Animals and treatment

All rats used in this experiment were male Fischer 344 from Hilltop Labs., Scottdale, PA, weighing approximately 300 g. After arrival, the animals were conditioned for one week. They were randomly divided into four groups ($n=4$) and treated respectively as follows: Group 1 (Sal) was pretreated with i.p. injections of saline on days 1, 3, and 5, then on day 6, intratracheally instilled with saline; Group 2 (Si) was pretreated with i.p. injections of saline on days 1, 3, and 5, then on day 6, intratracheally instilled with silica; Group 3 (Sal + Dex) was pretreated with i.p. injections of dexamethasone (2 mg/kg) on days 1, 3, and 5, then on day 6, intratracheally instilled with saline; Group 4 (Si + Dex) was pretreated with i.p. injections of dexamethasone (2 mg/kg) on days 1, 3, and 5, then on day 6, intratracheally instilled with silica. With all groups, bronchoalveolar lavage was performed 24 h after silica or saline vehicle intratracheal instillations.

Instillation of silica

Each animal was anesthetized with sodium methohexital (0.7 ml, 10 mg/ml) and suspended by the maxillary incisors on a wire. The tongue was moved and the larynx was illuminated using a modified laryngoscope. The silica particles were prepared at a concentration of 20 mg/0.5 ml saline. The 0.5 ml were instilled into the trachea by a syringe with a needle with a ball at the end. In control groups the same volume (0.5 ml) of saline vehicle was instilled.

Bronchoalveolar lavage (BAL) procedure and cellular differential

Twenty-four hours after the instillations, rats were anesthetized with 0.7 ml of sodium pentobarbital (64.8 mg/ml) and exanguinated by cutting the abdominal aorta. A lavage tube (PE 160) was placed into the trachea. The chest cavity of each animal was opened, and the large lobe of the right lung was tied off. The cells from the remaining lobes of the lung were collected by BAL. While massaging the lungs, 2 ml/100 g body weight of calcium- and magnesium-free Hanks' balanced salt solution (HBSS) was instilled into the lungs. For the first instillation, HBSS was allowed to remain in the lungs for 30 s, then withdrawn. The same buffer was reinstilled for another 30 s, then removed and saved for analysis in a separate tube. Lavage continued with additional aliquots filling an additional centrifuge tube (50 ml) for each rat. After lavage, both tubes for each animal were centrifuged at $500 \times g$ for 7 min. The supernatant solution from the first lavage fluid was frozen for later analysis of total protein. All pellets were combined for each rat while discarding the remaining supernatant fluids. Cells were recentrifuged and the pellet resuspended in 1.0 ml HBSS for cell counting. The cells were counted via a hemacytometer. For cell differentiation, cells (1.5×10^5) were spun

in a Shandon cytocentrifuge at 400 rpm for 4 min and stained with Wright-Giemsa stain. Alveolar macrophages, neutrophils, and lymphocytes were counted.

Luminol-dependent chemiluminescence (LDCL)

LDCL was measured with a Berthold LB9505C Luminometer (Wildbad, Germany). LDCL was followed for 20 min at 37 °C. The integrated response was determined with a KINB program supplied with the luminometer. Data are presented as cumulative counts per 20 min.

Cellular LDCL of bronchoalveolar cells: BAL cells were recovered from the lungs as described above. The cell number was adjusted to 10^7/ml. Luminol was used as an amplifier of the chemiluminescence. It was first dissolved in DMSO and then diluted in physiological HEPES buffer (0.1 *M*, pH 7.4). The final concentration of the luminol in the reaction mixture was 10^{-5} *M*. Phorbol myristate acetate (PMA) was used as a cellular stimulant at a final concentration of 10^{-5} *M* in the reaction mixture. The final volume in each cuvette was 500 µl (100 µl of cells, 100 µl of luminol, 100 µl of PMA, and 200 µl of HEPES buffer). Inhibitors of LDCL were also used in some of the reactions. Superoxide dismutase (SOD) or *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME) was added in a volume of 100 µl in place of 100 µl of HEPES buffer. The final concentrations were 1 mg/ml for the SOD and 1 m*M* for the L-NAME. When these inhibitors were used, they were incubated with the cells for 10 min at 37 °C before stimulation with the PMA.

Nonlavaged chopped lung LDCL: The lobes of lungs that were tied off were surgically removed after lavage. The lungs were placed on a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., Brinkmann, Westbury, NY). The tissue micrometer was set at 5.0, which produced slices about 0.5 mm thick, and the lungs chopped. The base was turned 90°, chopped, and repeated again. The cut lung portions (75 mg) were placed in luminometer cuvettes containing HEPES buffer (225 µl), luminol (100 µl), and PMA (100 µl). When inhibitors (SOD; L-NAME) of the light reaction were studied, 100 µl of each was used in place of an equal volume of HEPES buffer, and they were incubated for 10 min at 37 °C with the lung tissue samples before the PMA was added. The final concentration of PMA and luminol was 10^{-5} *M*, and the final concentrations for the inhibitors in the cuvette were 1 mg/ml for the SOD and 1 m*M* for the L-NAME.

Biochemical measurement

Total protein was measured in the first aliquot of the acellular BAL fluid by the method of Hartree [19] using bovine serum albumin as the standard. The measurement of total protein in the BAL fluid was used to quantitate the permeability of the bronchoalveolar–capillary barrier.

Statistics

An analysis of variance (ANOVA) was used to evaluate the data. If a significant interaction among the treatment groups was present, the *post hoc* Student–Newman–Keuls test was performed to determine which groups were significantly different from each other. The criterion for significance was $P<0.05$.

Results

Instillation of silica led to an increase in neutrophils and lymphocytes in BAL fluid, but not in alveolar macrophages (Fig. 1). These increases were prevented by dexamethasone treatment.

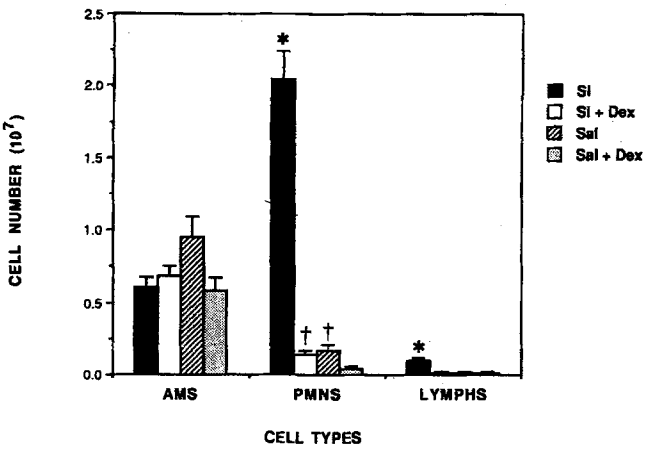

Fig. 1. The differentials of BAL cells recovered from the lungs 24 h after the intratracheal instillation of silica or saline vehicle. Rats received i.p. injections of either dexamethasone (Dex; 2 mg/kg) or saline (Sal) on days 1, 3, and 5. On day 6, one-half of the animals in these two treatment groups were then intratracheally instilled with either silica (Si; 20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). Alveolar macrophages (AMS), neutrophils (PMNS), and lymphocytes (LYMPHS) were counted. The error bars represent the standard error of the mean from four individual animals. The mean values of the number of PMNS and LYMPHS recovered from the Si group were significantly greater than the values of the other three groups (*$P<0.05$). The mean value of the number of PMNS recovered from the Si + Dex and Sal groups was significantly greater than the mean value of the Sal + Dex group (†$P<0.05$).

In the analysis of the total protein content of the acellular lavage fluid (Fig. 2), the animals in the Si group had a significant elevation in the protein levels when compared to the other three groups. The pretreatment of the animals with dexamethasone (Si + Dex group) prevented the leakage of protein into the airways and airspaces of the lungs caused by the silica exposure.

When the PMA-stimulated LDCL of the lung tissue recovered from the four groups of animals was measured (Fig. 3), the animals in the Si group had a significant elevation in the LDCL response when compared to the other three groups. This silica-induced elevation in the production of light was completely inhibited by the pretreatment with dexamethasone. The same pattern was demonstrated when BAL cells were subjected to PMA-stimulated LDCL (Fig. 4). A significant increase in LDCL was also observed in the animals from the Si + Dex and Sal groups when compared to the Sal + Dex group.

SOD, which catalyzes the breakdown of superoxide anion, and L-NAME, a nitric oxide synthase inhibitor, inhibited the LDCL response of the recovered BAL cells and lung tissue (Table 1). When SOD was preincubated with the lung tissue for 10 min, a nearly complete inhibition of the light reaction was seen in the Si group, while the inhibitory effect on lung tissue LDCL of the Si + Dex group was less pronounced. The inhibitory effect of SOD on BAL cell LDCL was similar for the Si and Si + Dex groups, but lower than with lung tissue. When L-NAME was preincubated with the lung tissue or BAL cells for 10 min, a significantly greater percent of inhibition of LDCL was observed in the Si group when compared with the Si + Dex group.

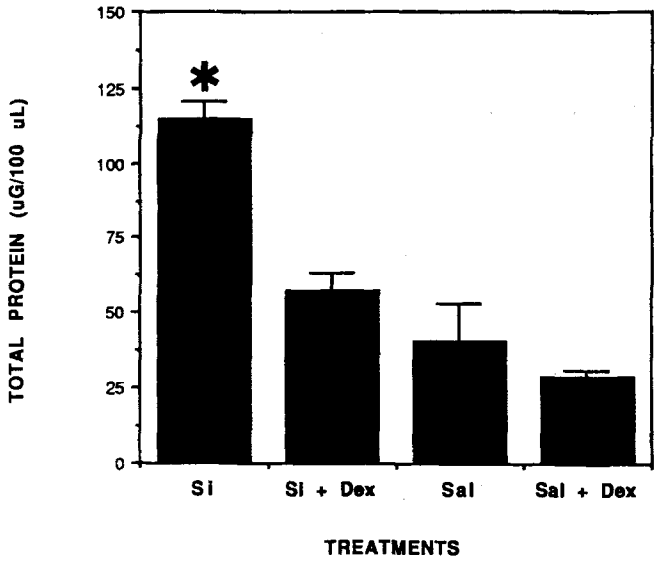

Fig. 2. Total protein present in the bronchoalveolar lavage fluids recovered from the lungs 24 h after the intratracheal instillation of silica or saline vehicle. Rats received i.p. injections of either dexamethasone (Dex; 2 mg/kg) or saline (Sal) on days 1, 3, and 5. On day 6, one-half of the animals in these two treatment groups were then intratracheally instilled with either silica (Si; 20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). The error bars represent the standard error of the mean from four individual animals. The mean value of the total protein recovered from the Si group was significantly greater than the mean values of the other three groups (*$P<0.05$).

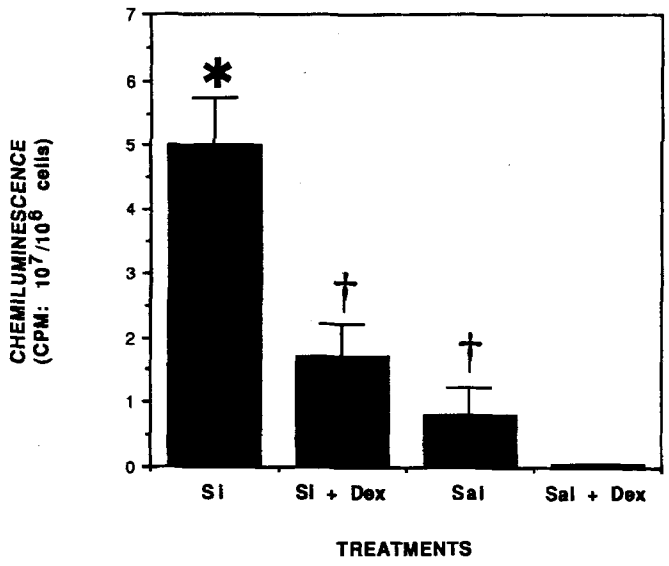

Fig. 4. The PMA-stimulated chemiluminescence of BAL cells recovered from the lungs 24 h after the intratracheal instillation of silica or saline vehicle. Rats received i.p. injections of either dexamethasone (Dex; 2 mg/kg) or saline (Sal) on days 1, 3, and 5. On day 6, one-half of the animals in these two treatment groups were then intratracheally instilled with either silica (Si; 20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). The error bars represent the standard error of the mean from four individual animals. The mean value of the chemiluminescence of the Si group was significantly greater than the mean values of the other three groups. (*$P<0.05$). The mean values of the chemiluminscence of the Si+Dex and Sal groups were significantly greater than the mean value of the Sal+Dex group (†$P<0.05$).

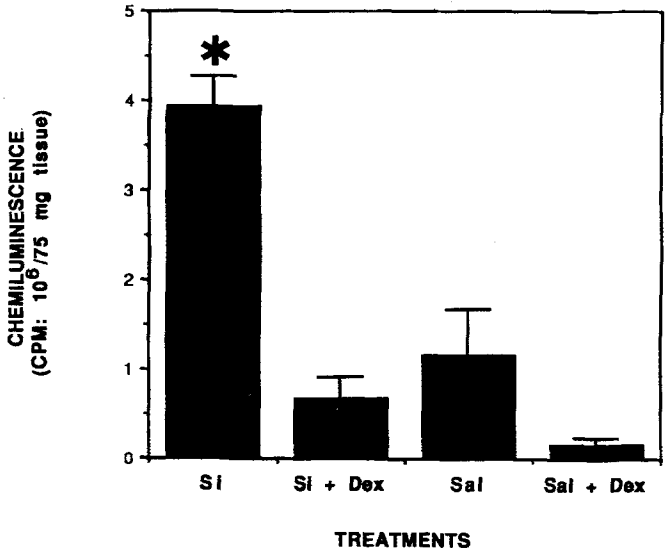

Fig. 3. The PMA-stimulated chemiluminescence of chopped, nonlavaged lung tissue recovered from the lungs 24 h after the intratracheal instillation of silica or saline vehicle. Rats received i.p. injections of either dexamethasone (Dex; 2 mg/kg) or saline (Sal) on days 1, 3, and 5. On day 6, one-half of the animals in these two treatment groups were then intratracheally instilled with either silica (Si; 20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). The error bars represent the standard error of the mean from four individual animals. The mean value of the chemiluminescence of the Si group was significantly greater than the mean values of the other three groups (*$P<0.05$).

Table 1. Percent inhibition of PMA-stimulated chemiluminescence

	SOD	L-NAME
Lung tissue		
Si	98.6±0.9*	52.2±3.4*
Si+Dex	70.7±7.6	7.6±4.9
BAL cells		
Si	55.8±5.8	63.3±2.2*
Si+Dex	64.0±3.2	26.8±2.2

Note. Rats received i.p. injections of either dexamethasone (Dex; 2 mg/ml) or saline (Sal) on days 1, 3, and 5. On day 6, one-half of the animals in these two treatment groups were then intratracheally instilled with either silica (Si; 20 mg/0.5 ml saline vehicle) or saline vehicle (0.5 ml). The percent inhibition of the PMA-stimulated chemiluminescence caused by SOD and L-NAME was measured on the lung tissue and BAL cells recovered from the animals of the treatment groups. The mean value of the Si group is significantly greater than the mean value of the Si+Dex group (*$P<0.05$).

Discussion

In an interesting clinical case [20], a silica-exposed open pit miner was treated with anti-inflammatory corticosteroids after exposure to the dust and experienced dramatic improvement in inflammation to his lung. It appears that early and continual treatment with steroids could be lifesaving in lung conditions where silica inhaled

material causes major lung toxicity. It then was our goal to determine the possible mechanism by which steroids reduce the lung damage caused by silica exposure. Using LDCL, we also investigated the role of peroxynitrite in the process of silica-induced inflammation.

In this study, 24 h after the intratracheal administration of silica, a profound inflammatory response in the lungs of rats resulted. This dramatic acute inflammatory response could be significantly reduced in silica-exposed animals pretreated with dexamethasone. The silica-induced pulmonary inflammation was demonstrated by the influx of neutrophils and an elevation of total protein in the BAL fluid. Previous studies have indicated that analysis of the BAL fluid is a sensitive means of characterizing acute inflammatory responses within the lungs [21–23]. Additionally, this inflammatory response to silica was observed by measuring the LDCL in chopped lung and BAL cells recovered from exposed animals. The experiments using the chopped, nonlavaged lung preparation represent the first time such a method has been reported for lung tissue which has been inflamed by an external stimulus such as silica. Chopped lung LDCL has the advantage of allowing an assessment of the inflammatory state of the tissue, and since all of the inflammatory cells cannot be removed by the lavage procedure, chopped lung LDCL may be a more complete measure of inflammation than cellular LDCL. In addition, chopped lung LDCL may be a more physiological means to study inflammation when compared with other cellular and biochemical indices assessed in the BAL fluid. Chopped lung LDCL permits a quantitative assessment of the ongoing inflammatory reaction, while the cellular differentials and biochemical measurements are static reflections of the source and results of the inflammatory process. In this study, all measures of inflammation correlated well.

The instillation of the saline vehicle alone resulted in a slight inflammatory response as demonstrated by the presence of neutrophils in the BAL and by LDCL of BAL cells. It is not surprising that dexamethasone inhibited this response.

The inhibition of LDCL using L-NAME, an inhibitor of NO synthase, suggests that a portion of this silica-induced oxidative response results from the production of NO [24]. It is known that superoxide and NO combine to produce peroxynitrite, which reacts with the luminol to produce LDCL [25]. The L-NAME was a more effective inhibitor of cellular and chopped lung LDCL when the animals were not pretreated with dexamethasone. Since dexamethasone has been shown to block the induction of NO synthase [18], the L-NAME then would be less effective in terms of its ability to inhibit the LDCL produced by peroxynitrite.

In conclusion, we have demonstrated that treatment of rats with an anti-inflammatory drug can prevent the acute inflammatory reaction to silica. The results described in the present study indicate that steroids can inhibit the acute tissue injury induced by silica. This effect may, in part, be mediated by inhibition of the induction of NO synthase. Further studies are underway to determine if the later silica-induced fibrotic response is also reduced by steroid treatment.

Acknowledgement. We gratefully acknowledge the financial support provided by the Generic Dust Center, US Bureau of Mines Grant 1195142-5423 and by the NIOSH Cooperative Agreement Program for Occupational Respiratory Disease and Musculoskeletal Disorders U60/CCU306149-03.

References

[1] M. Cherniak, The Hawk's Nest Incident: America's Worst Industrial Disaster, pp. 1–194, New Haven Press, Yale University 1986.

[2] M. R. Becklake, *Evaluation of respiratory hazards in the working environment through environmental, epidemiologic and medical surveys.* In *VIII International Pneumoconioses Conference*, Part I, pp. 16–21, 1988.

[3] D. Bowden, *Macrophages, dust, and pulmonary diseases.* Exp. Lung Res. *12,* 89–107 (1987).

[4] T. Gordon and D. O. Amdur, *Toxic responses of the lung.* In *Casarett and Doull's Toxicology,* 4th Edition. (Eds. M. O. Amdur, J. Doull and C. D. Klaassen) pp. 383–406, Pergamon, New York 1991.

[5] J. H. Vincent, D. Mark, A. D. Jones and K. Donaldson, *A rationale for assessing exposure – Dose-response relationships for occupational dust-related lung disease.* In *VIIth International Pneumoconioses Conference*, Part I, pp. 151–157, 1988.

[6] R. C. Lindenschmidt, K. E. Driscoll, M. A. Perkins, J. M. Higg, J. Maurer and K. A. Karl Belfiore, *The comparison of a fibrogenic and two non-fibrogenic dusts by bronchoalveolar lavage.* Toxicol. Appl. Pharmacol. *102,* 268–281 (1990).

[7] K. Van Dyke, J. Gutierrez, C. Van Dyke and C. Nowack, *"Piggy back" mechanism with calcium explain how silica exerts its toxicity to phagocytic cells.* Microchem. J. *48,* 34–41 (1993).

[8] B. Hille, Ionic Channels of Excitable Membranes, pp. 83–113, Sinairer Assoc. Inc., Sunderland, MA 1991.

[9] M. D. Englen, S. M. Taylor, W. W. Laegried, H. D. Ligitt, R. M. Silflow, R. G. Breeze and R. W. Leid, *Stimulation of arachidonic acid metabolism in silica-exposed alveolar macrophages.* Exp. Lung Res. *15,* 511–526 (1989).

[10] G. S. Davis, *Pathogenesis of silicosis: Current concepts and hypothesis.* Lung *164,* 139–154 (1980).

[11] W. N. Rom, P. B. Bitterman, S. I. Pennard, A. Cantin and R. G. Crystal, *Characterization of the lower respiratory tract inflammation of non-smoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts.* Am. Rev. Respir. Dis. *136,* 1429–1434 (1987).

[12] Y. Sibille and H. Y. Reynolds, *State of the art: Macrophages and polymorphonuclear neutrophils in lung defense and injury.* Am. Rev. Respir. Dis. *141,* 471–501 (1990).

[13] B. J. Stuebs, H. J. Cho, N. S. Kwon, M. F. Weise and C. F. Nathan, *Purification and characterization of the cytokineinduced macrophage nitric oxide synthase: An FAD and FMN containing flavoprotein.* Biochemistry *88,* 7773–7777 (1991).

[14] W. C. Sessa, J. K. Harrison, D. R. Luthin, J. S. Pollock and K. R. Lynch, *Genomic analysis and expression patterns reveal distinct genes for endothelial and brain nitric oxide synthase.* Hypertension *21,* 934–938 (1993).

[15] M. Sherman, E. E. Aeberhard, V. Z. Wong, J. M. Griscavage and L. J. Ignarro, *Pyrrolidine dithiocarbamate inhibits induction of rat nitric oxide synthase in rat alveolar macrophages.* Biochem. Biophys. Res. Commun. *191,* 1301–1308 (1993).

[16] R. Radi, J. S. Beckman, K. Bush and B. A. Freeman, *Peroxynitrite oxidation of sulfhydryls.* J. Biol. Chem. *266,* 4244–4250 (1991).

[17] J. J. Moreno and W. A. Pryor, *Inactivation of alpha-1-proteinase inhibitor by peroxynitrite.* Chem. Res. Toxicol. *5,* 425–431 (1992).

[18] T. McCall, R. M. J. Palmer and S. Moncada, *Induction of nitric oxide synthase in rat peritoneal neutrophils and its inhibition by dexamethasone.* Eur. J. Immunol. *21,* 2523–2527 (1991).

[19] E. Hartree, *Determination of protein: A modification of the Lowry method that gives a linear photometric response.* Anal. Biochem. *48,* 422–427 (1972).

[19] J. J. Denny, W. D. Robson and D. A. Irwin, *The prevention of silicosis by metallic aluminum.* Can. Med. Assoc. J. *37,* 1–11 (1937).

[20] G. B. Goodman, P. D. Kaplan, I. Stachura, V. Castranova, W. H. Pailes and N. L. Lapp, *Acute silicosis responding to corticosteroid therapy.* Chest *101,* 366–370 (1992).

[21] R. Henderson, *Use of bronchoalveolar lavage to detect lung damage.* Environ. Health Perspect. *56,* 112–129 (1984).

[22] M. F. Khan and G. S. D. Gupta, *Cellular and biochemical indices of bronchoalveolar lavage for detection of lung injury following insult by airborne toxicants.* Toxicol. Lett. *58,* 239–255 (1991).

[23] K. Van Dyke, *Introduction to cellular chemiluminescence: Neutrophil, macrophages and monocytes.* In *Cellular Chemiluminescence.* (Eds. K. Van Dyke and V. Castranova) Vol. I, pp. 3–24, CRC Press, Boca Raton, FL 1987.

[24] J. F. Wang, P. Komarov, H. Sies and H. DeGroot, *Contribution of nitric oxide synthase to luminol dependent chemiluminescence by phorbol ester activated Kupffer cells.* Biochem. J. *279,* 311–314 (1991).

[25] R. Radi, T. P. Cosgrove, J. S. Beckman and B. A. Freeman, *Peroxynitrite-induced luminol chemiluminescence.* Biochem. J. *290,* 51–57 (1993).